was stained with propidium iodide. The image of microtubules and chromatin was captured using laser scanning confocal microscope.

Result: In germinal vesicle stage oocyte, sperm chromatin remained condensation and sperm derived microtubules were not observed at 8 to 10 h after sperm injection. At 18~20 h after injection, the sperm nucleus developed to the metaphase chromatin along the metaphase structure of female nucleus. In some metaphase I stage oocytes, sperm chromatin decondensed at 8 h to 10 h after injection, sperm aster was seen soon after sperm injection. At 18~20 h after sperm injection into metaphase I stage oocyte, male chromatin developed to the metaphase chromatin while female chromatin extruded first polar body and formed the metaphase chromatin. At 18 to 20 h after sperm injection into preactivated oocytes, condensed sperm nucleus was located in close proximity of female pronucleus. However, the condensed nucleus did not fuse with female pronucleus. In preactivated ocytes, injected sperm remained condensation, a few sperm organized small microtubular aster. Instead, maternal derived microtubules were organized near the female chromatin, which seem to move condensed male chromatin near to the female pronucleus.

Conclusion: Sperm nuclear decondensing activity and microtubule organization activity of centrosome during fertilization are cell cycle dependent. In absence of male functional centrosome, female origin centrosome takes over the role of microtubule nucleation for nuclear movement.

B-14 Effect of Spermatogenesis by L-type and T-type Ca^{2+} Channel Blocker in Mice

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Introduction: Spermatogenesis is a complex and highly coordinated process by which spermatogonia proliferate and differentiate to produce mature sperm. There are ample evidences indicating that elevations of intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) are key signals for spermatogenesis, although little is known about its regulation in spermatogenic cells. A functional low voltage-activated Ca channel is expressed during the meiotic and post-meiotic stages of mammalian spermatogenesis. Pharmacological and physical analyses suggest that a Ca channel operation is required for actual spermatogenesis. This study was conducted to investigate the effect of L-type or T-type calcium channel blocker on in vivo spermatogenesis in mice.

Materials and Methods: ICR male mice of 18 day old were used. Nifedipine, a L-type Ca^{2+} channel blocker, was administered orally for 20 days at dosages of 20, 40 or 80 mg/kg body weight. Ethosuximide (Sigma, USA), a T-type Ca^{2+} channel blocker, was administered at 100, 500 or 1000 mg/kg body weight. Dosages were within the range of the general physiological treatment. The control group was fed with distilled water. After 20 days, spermatogenesis was investigated by measuring the size of testis and number of sperm in seminiferous tubules. The slides were stained with H/E staining following the standard fashion for pathological evaluation. Serum concentrations of LH, prolactin and testosterone were analyzed by radio-

immunoassay. Immunohistochemical staining was performed using a commercial kit. A rabbit polyclonal antiserum against transition nuclear proteins (TP2) was kindly donated by Dr Kistler, W. S., The TP2 proteins were known to be specific to the early elongated spermatid of rodent and thus used as a maker for the differentiation of spermatogenic stages.

Results: There was no difference of body weight and organ development between experimental and control groups of mice. However, the testis size of the mice treated with either 80 mg/kg of nifedipine or 1,000 mg/kg ethosuximide was reduced by about 25% compared to the control group mice. Testes of the control group mice showed all kinds of spermatogenic cells including elongated spermatid and mature sperm. However, mice treated with either one of the drugs exhibited that spermatogenesis was arrested mostly at primary or secondary spermatocyte stage and that seminiferous tubules developed poorly. Hormone assay results showed that blood prolactin level of drug-treated mice was not significantly different from that of the control group mice, while LH level of drug-treated mice was a little elevated compared to the control. Interestingly, testosterone level of treated mice dramatically decreased as compared to the control. To confirm the stage-specific inhibitory effect of the drugs, TP2 expression and its cellular specificity was examined immunohistochemically in testes of mice treated with the drugs or not. When the testes of the control group mice were immunostained with antiserum against TP2, positive immunoreactivity was found exclusively in early-elongated spermatids. Strikingly, testes of the mice treated with either one of the drugs showed also distinct immunoreactivity, mostly in round spermatids.

Conclusion: Based upon these studies, it is concluded that both T-type and L-type Ca channel play important roles in the development of mouse testis, particularly, seminiferous tubules. The impairment of either one type of calcium channel might result in the failure of the tubular development, which eventually leads to a blockade of spermatogenesis.

B-15 Cellular Distribution and Organization of Centrosomal Components, *NuMA* and *Nek2* during Mouse Spermatogenesis and Fertilization

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1. Aims

It has been well known that centrosomes are not present in the mouse oocyte, and that they appear during morula or blastocyst stage. However, the cellular distribution and organization of the centrosomal components in the oocyte is completly unknown during fertilization and embryonic development. We examined distribution of two well-known centrosomal proteins, *NuMA* and *Nek2* in mouse gametes to get an insight in the reorganization of centrosomal proteins during germ cell development and early fertilization.

2. Materials and Methods

Spermatogenic cells and oocytes were stained by immnocytochemistry to visualize distribution and be-