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-

havior of *NuMA* and *Nek2* proteins.

3. Results

Mitotically or meiotically active spermatogenic cells, spermatogonia or spermatocytes were intensively stained with anti-*NuMA* or anti-*Nek2* antibodies in both centrosomes and cytoplasm in spermatogenic cells, whereas the oocytes showed different staining patterns depending on the meiotic stages. In germinal vesicle oocyte, *NuMA* was present in the nucleus, and was very diluted over the ooplasm as maturation progresses. Only a few discrete spots were detected in mature oocyte at metaphase. *Nek2* was not possible to detect due to either low abundance or cell cycle-specific presence.

4. Conclusion

Spermatozoa appear to lose the two antigens during spermatogenesis. However, the oocyte has small amount of these antigens which may be involved in the reorganization of centrosomal components during early developement.

B-16 Effect of Hypo-osmotic Swelling (HOS) Test on Subsequent Post-thaw Testicular Spermatozoa

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Objective: Fertilization and pregnancies have been reported after ICSI using testicular sperm extracted from frozen-thawed seminiferous tubule. Immediately after thawing, however, testicular sperm do not show motility. Therefore, we studied the viability of immotile sperm extracted from frozen-thawed seminiferous tubule using hypo-osmotic swelling (HOS) test and eosin-Y test.

Materials and Methods: After testicular sperm extraction for ICSI, the remaining sections of seminiferous tubules were frozen with a computerized cell freezer. For the preparation of frozen-thawed testicular sperm, the seminiferous tubules were thawed by removing from LN_2 and leaving them at room temperature for 10 min followed by 37℃ water bath for 10 min. The prepared tubules were washed to remove the preservation medium. The sperm preparation method is similar as described previously. To evaluate the viability, frozen-thawed testicular sperm was suspended in 0.1 ml HOS solution three hours after *in vitro* incubation. Ten minutes later, the types of distally coiled sperm were assessed and defined as a positive reaction.

Results: In 44 cases of cryopreservation of seminiferous tubules in obstructive azoospermic patients, the fertilization rates with 2PN were 71.4% and pregnancy rates were 34.1%. The presence of motile spermatozoa on subsequent post-thaw testicular sperm was 15.1% and increased to 54% just before ICSI (three hours later *in vitro* incubation). Three hours after in *in vitro* incubation, the percentages of presence of motile sperm, reaction of hypo-osmotic swelling test and viable sperm was 63.6% (28/44), 93.2% (41/44), and 77.3% (34/44), respectively.