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

Park YS[1], Lee HS[1], Lee SH[1], Lim CK[1], Koong MK[2] and Yun JM[3]

Laboratory of Reproductive Biology & Infertility[1], Department of Obstetrics and Gynecology[2], Department of Urology[3], Samsung Cheil Hospital & Women's Healthcare Center, Sungkyunkwan University School of Medicine, Seoul, Korea

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.

Conclusions: Immediately after thawing, the testicular sperm did not showed motility. Although motility was gained after incubation, many cases the sperm remained non-motile until optimal insemination time. However, *in vitro* incubation of frozen-thawed testicular sperm showed positive reaction. And presence of motile- and viable sperm remarked HOS test could be an alternative method for the selection of viable sperm for ICSI.

B-17 DNA Synthesis and Sperm Mitochondria in Porcine Oocytes Following Porcine and Mouse Sperm Injection

Lee YJ, Kim BK and Kim NH

Department of Animal Science, Chungbuk National University, Cheong Ju, Chungbuk, Korea

Objective: To get insight into the nature of foreign mitochondria and syngamy during mammalian fertilization we compared fate of sperm mitochondria, DNA synthesis, and synkayogamy in porcine oocytes following microinjection of porcine or mouse spermatozoon.

Methods: At 8~10 and 18~20 hour following sperm injection, pronuclear movement, sperm mitochondria, and DNA synthesis were imaged with propidium iodide, mitotracker, and BrdU under confocal laser scanning microscope.

Results: Intracytoplasmic injection of either porcine or mouse spermatzoon activated porcine oocytes without additional parthenogenetic stimulation. Foreign mitochondria in either mouse or porcine sperm midpiece were introduced into porcine oocytes following sperm injection, but rapidly disappeared from the actively developing porcine oocytes. BrdU experiment showed new DNA synthesis in porcine oocytes following injection of mouse spermatozoon or sperm head. At 24 h after injection of mouse isolated sperm head or a spermatozoon, mitoic metaphase was seen in oocyte, but they did not go to normal cell division.

Conclusion: Pronuclear formation, foreign mitochondria disruption, DNA synthesis and synkayogamy during fertilization are not species specific processes.

B-18 생쥐 배반포기배의 초자화동결 · 초급속융해에 관한 연구

서울대학교 의과대학 산부인과학교실[1], 의학연구원 인구의학연구소[2]

성기청[2] · 김희선[2] · 오선경[1,2] · 서창석[1,2] · 김석현[1,2] · 최영민[1,2]
김정구[1] · 문신용[1,2] · 이진용[1]

목 적: 생쥐 배반포기배를 사용하여 초자화동결에 적절한 동결보존제와 방법을 확립하고자 하였다.

대상 및 방법: 6주령 F1 hybrid (C57BL♀×CBA♂)를 과배란 유도하여 체외수정시킨 후 수정이 확인