significantly higher rates of cytoplasmic fragment than those in pre-activation group. Fusion rates of cytoplasts and oocytes in preactivation group or normal NT group resulted in 67% and 78%, respectively. Also, their subsequent development into cleaved embryos (55% vs. 57%) and blastocysts (7% vs. 15%), respectively.

Conclusion: This result suggested that MVC method was appropriate freezing method for the bovine eMII oocytes and that vitrified eMII oocytes after pre-activation can support in vitro embryonic development after SONT as equally well as fresh oocytes.

P-18 Effects of Growth Factor on the Spermatogenic Cells from Infertile Men in co-culture System with TM4 Monolayer

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Objectives: The development of assited reproduction technique significantly improved pregnancy rate in male infertility. However, the problem of severe idiopathic male infertility were not solved inspite of many efforts. *In vitro* culture of spermatogenic cells were then initiated in an effort to try to overcome the low clinical outcome. Recently, the production of flagellar growing spermatid by *in vitro* culture system was achieved. Also, auto/xenotransplantation technique of spermatogonial cell were developed. The establishment of spermatogenesis *in vitro* is very important for clinical outcome and understanding of molcular events in male reproductive organ. Therefore, we evaluated the effect of growth factors (Stem Cell Factor (SCF), Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF)) in spermatogenic cell co-culture system and estimate for proliferative abilty of encasulation culture system with Sertoli cell monolayer.

Materials & Methods: From Jan. until Oct. 2001, 19 obstructive azoospermic patients and 4 non-obstructive azoospermic patients entering IVF/ICSI programme were enrolled. Spermatogenic cells were co-cultured with or without empty zona pellucidae on TM4 Sertoli cell monolayer supplemented with different concentrations of SCF and GM-CSF up to 168 hrs. Different survival rates were compared by trypan blue exclusion test and Hoechst staining. In order to compare the proliferation and differentiation abilty in different conditions, immunocytochemical staining with anti-*c-kit* antibody was performed. The presence of early spermatogenic cell was confirmed by RT-PCR with *c-kit* primer.

Results: The survival rate in encapsulated system attenuated the time-dependent death rate than free culture system. The survival rate of spermatogenic cells from obstructive azoospermic patients were slightly higher than that of non-obstructive azoospermic patients at 168 hrs culture. Addition of growth factor in encapsulated co-culture system increased proliferation rates of spermatogenic cells. In this system, the most optimal concentrations of SCF, GM-CSF were 1 ng/ml, 1 ng/ml, respectly. Presence of early spermatogenic cells was confirmed by expression of *c-kit* transcript during all culture period.

Conclusions: The encapsulation of spermatogenic cells with zona pellucidae in co-culture system enhanced cell survival and proliferation rates probably by protecting from loss of germ cells and various damage during *in vitro* culture. The addition of growth factor in appropriate concentrations improved cell growth as well.

P-19 The Genetic Analysis of LH-subunit Gene in Non-obstructive Male Infertilite: The Genetic Analysis in Male Infertility

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Objectives: To investigate the genetic background of non-obstructive male infertility.

Materials and Methods: 95 non-obstructive male infertile patients; 75 azoospermia, 18 oligo-astheno-teratozoospermia (OAT) and 2 oligozoospermia patients were investigated for genetic background including karyotype, Y chromosomal deletion and three polymorphism sites of LH-subunit gene (Trp8Arg, Ile15Thr, and Gly102Ser).

Results: An abnormal karyotype was found in eleven of the azoospermia patients (11/75) and one of the OAT patients (1/18). Twelve percents of non-obstructive male infertility patients (11/95) have one or more deletions at 13 loci on Y chromosome. Gly102Ser variant of LH-subunit gene was not detected in either infertile or fertile men (0%, n=294). The frequency of double Trp8Arg and Ile15Thr heterozyotes was similar between fertile (14.5%, n=200) and infertile (11.8%, n=76) group with the exception of one homozygous mutation (Arg8 and Thr15) from azoospermia.

Conclusions: Three variants of LH beta gene (Trp8Arg, Ile15Thr, and Gly102Ser) may not be associated with male infertility. The biological effect of Arg8 and Thr15 mutations related to male infertility should be further investigated.