## P-25 Asthenozoospermia 환자의 ICSI 시행시 Pentoxifylline을 사용한 정자처리법이 임상 결과에 미치는 영향

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Intracytoplasmic sperm injection (ICSI) is now widely used for male factor treatment in human IVF-ET program. Although poor sperm parameters including count, motility and morphology has been reported not important in ICSI program, sperm motility is an prerequisite indicator to find viable spermatozoa in the treatment of severe asthenozoospermia. It was shown that pentoxifylline (PF), being a phosphodiesterase inhibitor, increase sperm kinematic parameters and the number of spermatozoa exhibiting hyperactivated motility by raising the intracellular content of cAMP, a molecule involved in the generation of sperm energy. The study aim was to evaluate the effect of PF on the conventional ICSI program undergone in severe asthenozoospermia. Total 348 cycles of ICSI programs undertaken at CHA General Hospital from January, 1996 to September, 2000, were divided into two groups - injected with PF-treated sperm (PFT, 204 cycles) or non-treated sperm (NPFT, 144 cycles) and the clinical results of PFT group were compared with those of NPFT. PF-treatment on sperm increased their motility of normozoospermia and severe asthenozoospermia. Fertilization rate of PFT group was higher than those of ICSI programs undertaken using sperm of NPFT (69.7% vs. 62.9%, p<0.01). And, ET and clinical pregnancy rates of PFT were slightly higher than those of NPFT (93.1%, 44.2% vs. 90.3%, 36.2%). These results showed that treatment of PF has a beneficial role on selection of viable sperm in severe asthenozoospermia.

## P-26 Interactions of Rbm, a Male Infertility Protein, with hnRNP K and Tra2α Suggest its Function in mRNA Processing during Spermatogenesis

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**Objectives:** A tight correlation between male infertility and microdeletions in Y chromosome revealed the presence of a group of male infertility genes in the deleted loci. However, functions and mechanisms of these genes on male infertility have not been clearly understood yet. To have an insight on biological functions of RBM, a male infertility gene in Y chromosome, we tried to identify proteins that interact with RBM.

Materials and Methods: Protein-protein interactions were investigated using the yeast two-hybrid interaction method.

Results: In a yeast two-hybrid system, Rbm appeared to interact with hnRNP K, whose functions are related to controlling splicing, transcription and signal transduction. In addition, we also observed a specific

interaction of Rbm with  $Tra2\alpha$ , whose function is in relation to RNA splicing. Interestingly, interaction between  $Tra2\alpha$  and hnRNP K was also observed. In transfected tissue culture cells, Rbm and hnRNP K appeared to be co-localized in the nucleus.

Conclusions: Interactions of Rbm with hnRNP K and Tra2 $\alpha$  suggest its function in RNA-splicing during spermatogenesis. In fact, it was well documented that a number of transcripts are spliced alternatively in male germ cells in developmental stage-specific manner and play critical functions in spermatogenesis. Further studies to confirm Rbm interactions in vivo are in progress.

## P-27 The Effect of Blocking of Occludin on Blastocoel Formation and Trophectoderm Differentiation in Mouse Preimplantation Embryos

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Tight junction (TJ) formation is critical for blastocoel formation in the mammailan embryos. Occludin, one of the TJ molecules, assembly occurs at late morula stage and leads to the establishment of a permeability seal to maintain the integrity of the cavitating blastocyst. In this study, the role of occludin on blastocyst morphogenesis was verified in mouse preimplantation embryos. Early and late morula were cultured in KSOM in the presence or absence of 0.05% sodium azide (SA), mouse seurm (6  $\mu$ g/ $\mu$ l), and/or occludin antibodies (6  $\mu$ g/ $\mu$ l) for 24 h and 14 h, respectively. Blastocoel formation was observed in the embryos cultured in control medium and the medium containing 0.05% SA + mouse serum, but not in the medium containing occludin antibodies (early morula 0%, late morula 46%). Blastocoel formation rate decreased in the presence of occludin antibodies in a dose-dependent manner. Expression of trophectoderm-specific gene (h19) was examined in the cultured embryos by RT-PCR. h19 transcript was not detected in the embryos cultured in the medium containing occludin antibodies. These results suggest that occludin engages in the blastocoel formation and contributes trophectoderm differentiation and that h19 gene is expressed after blastocoel formation.