Results:

I) To investigate *in vitro* motility of frozen-thawed human sperm, when PF of various concentration prior to freezing was examined, the result of 5 mM treatment group (51.0%) was higher than those of the other treatment groups (control: 39.0; 2.5 mM: 40.0; 10.0 mM: 46.0%) ($p<0.01$).

II) In case of intact acrosome rate, FPP treatment group before freezing was higher than those after thawing. Especially, 50 nM (75.5%) FPP adding in all treatment procedures for human semen freezing (before freezing, freezing and after thawing) was significant effect on maintaining of the sperm intact acrosome (control: 45.0; 25 nM: 53.0; 100 nM: 68.0%) ($p<0.01$).

III) Based on these data, when the additive effects of PF and FPP on sperm motility and intact acrosome were compared simultaneously, intact acrosome rate in FPP treatment group (65.0%) was significantly higher than that in PF treatment group (43.0%) ($p<0.05$), although sperm motility was slightly higher in PF treatment group.

Conclusions: These results demonstrated that the more improved sperm fertilizing ability of frozen-thawed human sperm can be obtained by addition of 50 nM FPP in all semen freezing procedures.

P-14 Molecular Cloning of the Human Adam 6 Pseudogenes

삼성제일병원 발생생물학 및 불임연구실, [1]성균관대학교 의과대학 산부인과

이형송 · 박용석 · 강인수[1]

Objectives: The cell-cell interactions that occur between sperm and egg involve not only the binding but also the fusion of the gamete plasma membranes. Numerous studies have implicated several different molecules on both the sperm and egg as being involved in gamete membrane interactions. The sperm proteins that have received the most attention recently have homology to disintegrins, which are proteins in snake venoms. These sperm disintegrin-like proteins are members of a molecular family, known as ADAMs (a disintegrin and metalloprotease) or MDCs (metalloprotease, disintegrin and cysteine-rich). In this study, to identify further members of ADAM family in the testis, we have searched the expressed sequence tags (EST) section of the EMBL nucleotide database. ADAM-like EST sequences that were not identical to known ADAMs were further analyzed to determine whether they contained putative ADAM-specific sequences.

Materials and Methods: Using the similarity search of the EST database and RT-PCR we identified a partial cDNA clone that encodes the 3' end of a putative novel ADAM. One million independent recombinant bacteriophage from a human testis cDNA library in λTriplEx was screened using a human ADAM6 partial cDNA that had been isolated from human testis RNA by RT-PCR. And the 5' end was amplified by PCR and the full-length cDNA was cloned and sequenced. And DNA sequencing of PCR fragments using genomic DNA as template with *hADAM6p*-specific primers confirmed the DNA sequences.

Results: The novel human ADAM (*hADAM6p*) shows striking sequence similarity to other members of the MDC family, especially macaque (*Macaca fascicularis*) *tMDCIV* (*ADAM6*). DNA sequencing of *hADAM6p* showed that it was a processed pseudogene. The pseudo-coding regions of this gene contain all

of the domains found in ADAMs. But *hADAM6p* is a truncated form which terminates within the pro domain. This truncation apparently arises from a deletion of the one nucleotide (nt position 295), resulting in an in-frame termination codon 354~356. *hADAM6p* is most similar to macaque *tMDCIVa* with a 84.4% amino acid identity. A blast search of the available genomic databases revealed 5 different *hADAM6p*-like sequences distinct from *hADAM6p*. The nucleotide sequences of *hADAM6p*-like genes exhibit about 91~93% homology to *hADAM6p*.

Conclusions: Full-length clones have been isolated from human testis cDNA library corresponding to a 2522 bp mRNA. The *hADAM6p* shows striking sequence similarity to other members of the MDC family, especially macaque *tMDCIV* (*ADAM6*). But DNA sequencing of *hADAM6p* showed that it was a processed pseudogene. Further experiments to determine the sequence of the parental *hADAM6* transcript and the function of the *hADAM6p* and the *hADAM6*-like proteins are required.

P-15 산전 유전진단을 위한 소 할구에서의 PCR-RFLP 이용

고려대학교 자연자원대학원[1], 한국생명과학연구소[2], 고려대학교 생명환경과학대학[3]

김득중[1,2] · 서동삼[3] · 정구민[2] · 고 용[1,3]

본 연구는 한우의 체외수정 후 미세조작기를 이용하여 분리된 할구를 PCR-RFLP 기법을 이용하여 소의 성장호르몬의 유전자형을 분석하였으며, 이는 향후 체외수정을 통한 산전 유전병과 관련된 질병 검색에 이용할 수 있는 모델을 개발하기 위하여 실시하였다. 미세조작에 따른 수정란의 생존성을 알아보기 위하여 미세조작기를 이용하여 6~8 세포기배에서 1개의 할구를 분리한 후 체외배양하였던 바 처리군 (biopsy)의 배반포까지의 발생률은 45% (48/105)였으며, 대조군은 65% (59/90)로 나타났다. 할구를 이용한 bGH의 PCR 증폭도는 분리된 할구를 1개, 2개, 4개, 8개를 사용하였을 때 모두 bGH 유전자 768 bp 위치에서 bGH 유전자를 확인할 수 있었다. 단일 할구를 3회에 걸쳐 총 102개에 대하여 반복 PCR을 실시한 결과 74.5%에서 bGH 유전자가 증폭되었으며, 증폭을 확인할 수 없는 경우는 25.5%였다. bGH 유전좌위의 증폭산물에 대한 *Msp I*의 제한효소 처리 결과, bGH AA형은 612 bp, 93 bp, 63 bp의 단편을, AB형은 705 bp, 612 bp, 93 bp, 및 63 bp의 단편을, 그리고 BB형은 705 bp, 및 63 bp의 단편을 나타내었다. 본 연구에서는 한우 체외수정란의 단일 할구에서 성장호르몬 유전자를 PCR 기법을 이용한 후 제한효소로 처리하여 그 유전자형을 확인할 수 있었다. 따라서 PCR 증폭효율을 높인다면 체외수정 후 단일 할구를 이용한 산전 유전진단을 PCR 기법을 이용하여 진단할 수 있을 것이라 사료된다. 따라서 본 연구 결과는 산전에 유전질병과 관련된 유전자를 검색할 수 있다는 임상적인 가능성을 제시하였다.