cells. DNA was prepared by suspending single embryos in PCR lysis buffer containing 200μg/㎖ proteinase K and incubated at 50℃ for 1hr. Metaphase chromosome spreads were prepared from nocodazole-treated embryos by air-drying method. To eliminate possible false positive signals, two sets of bovine-and Y chromosome-specific primers were used in the PCR. Two amplified products (bovine- and Y-specific) were obtained in male samples whereas only one product (bovine-specific) in female. FISH and PRINS were used to identify the Y chromosome on metaphase spreads. The fluorescent Y-specific signal was stronger in PRINS than in FISH.

The results suggest that a rapid, accurate and efficient sexing is now possible in bovine preimplantation embryos produced *in vitro* using PCR. This was evidenced by PRINS.

P-6

Single Cell Analyses of Dystrophin Gene and Sexing Using Whole Genome Amplification

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Recently, developed primer extention preamplification(PEP) method amplifies the whole genome and simultaneous multiple DNA analyses became possible. It leads to the development of preimplantation genetic disease diagnosis using single cells from early embryo, sperm, polar body and oocyte. The most important advantage is the ability which can investigate several loci simultaneously and confirm results by analysing multiple aliquots for each locus. Whole genome from each single cells was amplified using 15-base oligonucleotide random primers. In this study, we performed PEP-PCR for applicate on prenatal and preimplantational genetic diagnosis in 20 cases of single amniocytes and 20 cases of single chorionic villi cells. We stuied 7 gene loci simutaneous analysis of single cells at two locus of exon 46, 47 and two VNTR(variable number tandem repeat) markers using 5'dysIII, 3'CA related to dystrophin gene and ZFY, alphoid repeat Y, DYS14 regions on chromosome Y. In all these cases, ninety seven percent of PEP reactions with single amniocytes and chorionic villus cells were successful. We obtained 38/40(95%) of accurate gender determination by comparing chromosome analysis and general PCR from gDNA. Therefore, these results have significant implications for a sperm or oocyte typing, prenatal and a preimplantational genetic diagnosis.

Group 2, discussion : 14:00~14:30

P-7

실패하였던 정관부고환문합술과 정관정관문합술에서 MESA-ICSI와 TESE-ICSI의 효용성

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연구목적 : 폐쇄성 무정자증의 치료방법인 정관부고환문합술과 정관수술후의 정관복원을 위한 정관정관문합술을 시행후 실패하였던 경우, 재수술을 시행할 때 수술의 성공에 대하여 절대적인 확신을 가질 수 없다. 그러므로 재수술을 할 때 부고환이나 고환에서 정자를 채취하여 난자의 세포질내로