IS-93 Possible role for TGF-a and EGF in the inflammatory mechanism of human ovulation

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[Objective] IL-8 and GRO-αhave been demonstrated to be important modulators of neutrophil-chemotaxis in the inflammation-like mechanism of human ovulation. In this study, we investigated the possible effects of TGF-$\alpha$and EGF on IL-8 and GRO-αsecretion by granulosa cells. [Methods]We used immortalized granulosa cells and granulosa-lutein cells with informed consents. Confluent cells were placed in serum-free media and treated with the above-mentioned growth factors for 8 hrs. IL-8 and GRO-αwere measured in the culture media using an ELISA. [Results] Treatment of immortalized granulosa cells with 10nM of TGF-$\alpha$and EGF produced higher levels of IL-8 than untreated cells by 8 hrs(241.8 ± 21.8, 261.4 ± 19.4 pg/ml ; control = 125 ± 15pg/ml ; $P<0.0001$). On the other hand, the same treatment produced slightly higher levels of GRO-αthan untreated cells by 8 hrs. Treatment of granulosa-lutein cells with 1nM of TGF-$\alpha$and EGF produced higher levels of IL-8 than untreated cells by 8 hrs(980.1 ± 51.5, 1134.6 ± 76.2 pg/ml ; control = 593.9 ± 30.2 pg/ml ; $P<0.0001$). Furthermore, the same treatment produced higher level of GRO-αthan untreated cells after 8 hrs($P<0.0005$). [Conclusion] Given the significant effects of both TGF-$\alpha$and EGF on IL-8 and GRO-αsecretion by granulosa-lutein cells, our data suggest that TGF-$\alpha$and EGF might play a role in the inflammatory mechanism of human ovulation.

IS-94 Quantification analysis approached to the duplication of dystrophin gene for prenatal and preimplantation genetic diagnosis

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[Objective] Duchenne muscular dystrophy(DMD)is alleric X-linked recessive disease caused by mutations in the dystrophin gene. Duplication type of mutations needs quantification analysis for diagnosis. We investigated to develop the quantification method for prenatal diagnosis and preimplantation genetic diagnosis(PGD). [Methods] 1)After DNA extraction from amniotic cells of carrier mother with exon 5—9 duplication, exon 6 and 11 from 100ng of the DNA were amplified by multiplex PCR, and then quantified by densitometer for prenatal diagnosis. 2)After DNA extraction from maternal peripheral blood and DNA dilution into 1ng, 500pg, 200 pg, 50pg, and 10pg, the DNA was amplified by multiplex nested PCR, and then each exon was quantified by Gene Scan analysis ; normal single amniotic cell was examined as controls. These examination was performed after informed consent was obtained. [Results] An intact boy was obtained by the result of the prenatal diagnosis with high probability(C.V. 0.1～0.4). With regard to PGD, the amplified two exons could be quantified to the extent of 50 pg of DNA by nested PCR, in which first cycle was 20 and second cycle was 33. The ratio of them is 1.5 fold in heterozygous female patients compared with normal amniotic cells. [Conclusion]It is suggested that the diagnosis of duplication is possible from single cell to carry out PGD by multiplex nested PCR.

IS-95 Development of preimplantation genetic diagnosis(PGD)for insertion and point mutation of Duchenne muscular dystrophy(DMD)

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[Objective] We have developed accurate PGD protocols of single gene deletion using multiplex nested PCR instead of gender determination for DMD. However, other mutations of single base insertion or point mutation need direct sequencing following nested PCR. The diagnostic accuracy was examined for PGD. [Methods] Nested PCR from single cell was performed from blood samples of normal men, women, and DMD patients(single base insertion/point mutation). All specimens were used under the informed consent. The following experiments were performed ; 1)most sensitive conditions for nested PCR to exon45 were investigated, 2)diagnostic accuracy was compared between gender determination using DYZ-1 and single gene amplification(exon45), and 3)diagnostic efficiency for DNA sequencing was examined using specimens of DMD patients. [Results] 1)The accuracy was 90%when the nested PCR condition was 30 cycles each for 1st/2nd PCR, and annealed at 64/62℃ for 60sec. 2)While DYZ-1 with thousands of repeated copies was amplified in all specimens from single cell, exon45 was amplified in 90%of cases. 3)The sequencing was successfully analysed in all specimens when nested PCR was successfully amplified. [Conclusion] It is suggested that the genetic diagnosis of single base insertion and point mutation is possible from single cell, and the diagnostic efficiency depends on the accuracy of nested PCR.