D-23 Screening of mutations in the dystrophin gene by PCR amplification of deletion-prone exon sequences

김언경[1*], 조민순[2], 채재진[1], 김성한[2], 배지현[1], 남궁용[3], 이정주[2]
서울대학교 분자생물학과[1], 서울대학교 생물학과[2], 강릉대학교 생물학과[3]

Duchenne Muscular Dystrophy (DMD) is a common X-linked neuromuscular disorder with an incidence of one in 3,500 male newborns. The disease is caused by a deficiency of the cytoskeletal protein, dystrophin as a result of mutations in the DMD gene. About two-thirds of DMD patients have a deletions of one or more exons, while one third is associated with smaller mutations, predominantly point mutations. In this study, we analyzed dystrophin gene from DMD patients by PCR amplification of selected deletion-prone exons and small mutations by SSCP analysis. We observed two mutations comprising a deletion that eliminated exon 50 and a nonsense mutation in exon 45, TCC(Ser$_{2172}$)→TTC(Phe). This nonsense mutation is a novel one that have not been reported anywhere.

D-24 Ultrastructural change of zona pellucida in mouse blastocyst during hatching

이정은*, 이동률[1], 김문규[1], 윤현수
영동제일병원 불임의학연구소, [1]한양대학교 생물학과

In mammal, the hatching of the blastocyst from zona pellucida(ZP) is prerequisite for implantation and further development. The hatching mechanism involves pressure exerted on the ZP by expansion of blastocyst and lysis of the ZP by substances elaborated either from the embryos or female reproductive tract. Recently, we reported that the supplementation of pronase E(PE) in culture media was not disturb embryonic development but increased the hatching rate in mouse. Therefore, we observed the ZP structure in *in vivo* blastocyst, *in vitro* cultured blastocyst and *in vitro* PE-treated blastocyst by scanning electron microscopy in order to evaluate hatching mechanism. We used ICR strain mice. *In vivo* blastocysts were obtained from uterus at 100 hours after hCG injection. The 2-cell embryos were obtained from oviduct at 48 hours after hCG injection and cultured in 0.5% BSA-HTF, *in vitro* blastocyst group, or in 0.5% BSA-HTF containing 1μg/ml PE, PE-treated blastocyst group, for 72 hours. The rate of development was compared and then the blastocysts were fixed in 0.5% glutaraldehyde in 0.1M cacodylate buffer, post-fixed in osmium tetroxide, dehydrated in alcohol series, critical point-dried with CO_2, sputter-coated with gold and examined in a scanning electron microscope. In *in vitro* blastocysts, the ZP appeared fenestrated spongelike structure. But, the ZP of *in vivo* blastocysts and PE-treated blastocysts appeared as if fenestrated spongelike structure wore away and to be thin. The hatching was accelerated in PE-treated blastocysts than in vitro blastocysts. From these results, we found that hatching *in vivo* was different from *in vitro*. We suggest that the uterine factor(s), assumed to be proteolytic enzyme(s), play an important role of hatching *in vivo*.