Z303 Relationship between Ca2+-ATPase Activity and Intracellular Calcium Level in Human sperm

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It has been known that during capacitation the influx of Ca^{2+} into the spermatozoan cytoplasm, and that in this period, the higher Ca^{2+} concentration of the extracellular fluid, the higher Ca^{2+} concentration of the intracellular fluid was kept to induce the capacitation and then finally to enter the acrosome reaction.

The present study was thus designed in order to examine a physiological role of Ca^{2+} which has been known as an essential factor for capacitation, to confirm whether the enzymatic activites of Ca^{2+}- ATPase on capacitation are important or not by means of Chlortetracycline(CTC) technique, and to clarify relationship between various levels of the Ca^{2+} concentration and the Ca^{2+}-ATPase which is known to be an important factor of the plasma membrane.

In the present study applying quercetin which has been known as an Ca^{2+}-ATPase inhibitor, the enzymatic effect of Ca^{2+}-ATPase on capacitation was found to be remarkable: a significant increase of the transformation from the original type to the B type and the AR type of spermatozoa. This finding suggests that Ca^{2+}-ATPase play an important role in the efflux and the influx of the Ca^{2+} which have been known to be an essential factor for the capacitation and acrosome reaction, and that the inhibitory action of the Ca^{2+}-ATPase might be a prerequsit step toward the acrosome reaction.

The conclusion reached can be introduced as follows: increment of the intracellular Ca^{2+} concentration occurred by controlling the slope of Ca^{2+} concentration through Ca^{2+}-ATPase activites in both the intra-and extra-cellular fluid may be an important procedure for capacitation and acrosome reaction, and finally for fertilization of the sperm and the ovum.

Z304 The morphogenesis of preimplantation mouse embryo is regulated by the oocyte derived-cytoplasmic factor in repressive manner

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This study was performed to investigate whether the morphogenesis is regulated by the oocyte derived-cytoplasmic factor and its repressive manner in preimplantation mouse embryo. A blastomere of 2-cell embryo was reconstructed with enucleated cytoplasm of fertilized oocyte(2+P group) and 2-cell embryo(2+2 group) by electrofusion. Also, karyoplast of 2-cell were fused with enucleated fertilized oocyte(2PP group). The development and morphogenesis of these reconstructive embryos was examined during cultivation. Number of cells was counted at the time of the early signs of compaction. Also, in these groups, the expression of ZO-1α$^+$isoform was analyzed by RT-PCR. In order to evaluate whether time schedule of embryonic morphogenesis is regulated by maternally derived factor or newly synthesized factor after fertilization, the half fertilized oocyte(both pronuclei residing) was reconstructed with half enucleated cytoplasm of fertilized oocyte which was cultured for 24h in the presence of cycloheximide(PP-CHX group), and their cell numbers at compaction stage and development were examined and compared with reconstituted embryos such as a half fertilized oocyte and enucleated cytoplasm of 2-cell embryo(P+2 group) or fertilized oocyte(P+P group). Embryonic development and gene expression of ZO-1α$^+$ isoform of 2+P and 2PP group was delayed when compared with those of the 22 group. Also, the phenomena of compaction and blastoceol formation were delayed in the development time and cell stage. The time and cell stage of compaction in embryo of PP-CHX group was similar to that in P+2 group. From these results, addition of pronuclear stage cytoplasm to 2-cell nucleus(blastomere) induced the delayed gene expression and early embryonic morphogenesis. And the inhibition of protein synthesis in the transferred cytoplasm was not affected to facilitation of morphogenesis in the reconstructed embryo. Therefore, we may suggest that oocyte derived-cytoplasmic factor(s) regulate(s) the early embryonic morphogenesis in repressive manner.