Z307 The Alterations of Meiotic Spindle Assembly during Ageing of Ovulated Oocytes in Mouse

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The alterations in the organization of meiotic spindle assembly were examined during ageing of ovulated, metaphase-arrested oocytes of ICR mice. The spindle assembly was investigated by immunohistochemistry of beta-tubulin and acetylated alpha-tubulin. Fresh ovulated mouse oocyte showed the compact anastral and barrel-shaped spindles located peripherally. Examination of mouse oocytes aged in vivo or in vitro showed an increased incidence of spindle abnormalities and of the proliferation of ooplasmic microtubules. The acetylated tubulin staining intensity of the aged mouse oocytes revealed stronger staining over the whole of the spindle than that of the fresh ovulated oocytes. It is concluded that abnormal incidence of meiotic spindle assembly correlates with the ageing of oocytes and the distribution of acetylated tubulin in the spindle of oocytes appears to be sensitive to oocyte age in mouse.

Z308 The Role of Genomic Methylation in Gene Expression and Development of Mouse Embryos

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To exam the role of genomic methylation in gene expression and development of mouse embryos, 5-azacytidine (5-azaCR) and 5-aza-2′-deoxycytidine (5-azaCdR) as DNA demethylating agents, and 6-azacytidine as a control were administered at 2-cell (47 hr post-hCG) stage in culture medium.

The development of 2-cell embryos was severely affected by the treatment of DNA methylation inhibiting agents which was dose dependent. The embryonic development of 2-cell embryos was not affected by the treatment of 6-azacytidine. The effects were may be caused by the incorporation of 5-azaCdR into DNA, but 5-azaCR is another pathway. The mRNA levels of *β-actin, c-myc,* or *hprt* genes were selectively changed on a time dependency scale, but the *c-fos* transcripts was not detected.

From these results, it was ascertained that the perturbation of adequte genomic methylation status that resulted from the incorporation of DNA and/or RNA of DNA methylation inhibiting agents may be affected on embryonic development by the changes of some gene expression which is sensitive to the alteration of genomic methylation status.