P-25 Spindle and Chromosome Configurations of in vitro Maturation Failure Metaphase I Arrested Oocytes Collected from PCO Patients

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Objectives: The in vitro maturation of immature oocytes retrieved from unstimulated ovaries has been interested in assisted reproductive technology. Oocyte maturation, characterized by germinal vesicle (GV) break-down, formation of first meiotic spindle, extrusion of the first polar body, and arrest in metaphase of the second meiotic division. However, numerous anomalies in the nuclear and cytoplasmic maturation were induced during in vitro maturation. This study was conducted to analyze spindle and chromosome configurations of in vitro maturation failure metaphase I (MI) arrested oocytes collected from PCO patients.

Materials and Methods: Immature oocytes were aspirated using ultrasound guided aspiration method with specially designed injection needle from unstimulated PCO patients. Oocytes with a germinal vesicle were cultured in maturation medium for 48 h. Oocytes were removed cumulus cells with hyaluronidase and mechanical pipetting after culture. Oocytes without an intact GV and 1st polar body were defined as metaphase I. Oocytes were used for immunostaining by using monoclonal anti-tubulin antibody and TRITC-conjugated second antibody

Results: Absent (85%) or disorganized (15%) spindle and dispersed chromosomes (100%) were observed in all analyzed oocytes

Conclusions: This result suggested that the MI arrested oocytes was related to the MI spindle, with absence of microtubules and dispersion of the chromosomes

P-26 Molecular Markers during the Onset of Implantation in the Mouse Luminal Epithelium

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Objectives: Implantation is defined as a process by which the blastocysts make the first physiological contact with the maternal uterine luminal epithelium. Blastocysts and uterus generate various factors during implantation, but it is likely that the molecular "cross-talk" between them involves many more yet unknown factors. Identification of essential regulatory factors is necessary to understand the basis for the establishment of pregnancy or the underlying causes of pregnancy failures. To address this issue, we isolated luminal

epithelium from implantation (IM) and interimplantation (INTER) sites using Laser Captured Microdissection (LCM) and analyzed the gene expression profiles by Microarray analysis.

Materials and Methods: ICR female mice were mated with fertile males to induce pregnancy (day 0.5 = vaginal plug). On day 4.5, uteri were divided to IM and INTER sites and immediately frozen with OCT compond, sectioned (5 μm), and stained with hematoxyline and eosin. RNA was extracted from captured cells, amplified with the RiboAmp RNA Amplification Kit, labeled and hybridizes to the Murine 6K GeneChip Expression Arrays (Digital Genomics, Seoul, Korea). Four replicates of hybridization were performed and results were statistically analyzed by Significance Analysis of Microarrays (SAM, Tusher et al., 2001).

Results: Comparison of IM and INTER sites by SAM identified 73 most highly ranked genes at IM, while 13 genes at the INTER sites, with an estimated false discovery rate (FDR) of 0.163. Differentially expressed genes were categorized based on the best available information regarding their biologic functions. Among 73 genes at IM, 20 were EST/unknown function, and the remain 53 were related to structure (24, 45.3%), metabolism (6, 11.3%), signal transduction (7, 13.2%), immune reaction (6, 11.3%), cell cycle (4, 7.5%), gene/protein expression (4, 7.5%), and oxidative stress (2, 3.8%). Of the 24 structural genes, 14 were related especially to expracellular matrix and tissue remodeling. Meanwhile, among 13 genes at INTER, 8 genes were EST/unknown function, and the rest 5 were related to metabolism (3), signal transduction (2), and gene/protein expression (1). Among these 58 (53+5) genes with known functions, 13 genes (22.4%) were related with Ca^{2+} for their function.

Conclusions: We demonstrated that gene expression profile of the IM and INTER can be successfully obtained with the small amount of purely isolated luminal epithelium by integrating the technologies of LCM with cDNA microarrays. Results of the present study revealed that 1) at the IM sites, active tissue remodeling is occuring during embryo apposition while the INTER sites are relatively quiescent than IM sites, and 2) the Ca^{2+} may be a vital regulatory factor for apposition process. Identification of unique gene expression profiles for the onset of implantation signifies that genome-wide analysis coupled with functional assays is a promising approach to resolve the molecular markers and pathways required for the successful implantation.

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P-27 The Effects of Yi-Jin Tang on Body Weight and Ovarian Reaction in Obesity Mice

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Objectives: In order to study the effect of Yi-Jin Tang (二陳湯) on body weight and ovarian reaction in obesity mice. We observed the effect of changes of body weight, ovulation rate, in vitro fertilization and early embryonic development of oocytes.