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Derivation of Oocyte-like Structure from Mouse Embryonic Stem Cells

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Oogenesis *in vitro* should contribute to various areas, including nuclear transfer and manipulation of the germ line, and advance studies on fertility treatment and germ and somatic cell interaction and differentiation. Embryonic stem (ES) cells are known as pluripotent and there are a few data in germ line cell differentiation from the ES cells. This study was carried out to examine whether three types of mouse (m) ES cells (D3, our own developed mES04 and Parthenogenetic-mES04) can develop into oogonia *in vitro*. To differentiate *in vitro* into oocytes, three types of ES cells were plated in tissue culture plate at a density of $1-2.5 \times 10^4$ cells/cm² using ES culture medium without LIF. Non-adherent cells were removed after 3-4 days and medium was replaced. Cultures were maintained for an additional 3 days. Upon further differentiation, cells were loosened off the plate and formed small aggregates in suspension. Ten primary follicle-similar structures among the aggregates were transferred into each well of 4 well dish and further cultured in MEM- α supplemented with 0.3% BSA, 0.23 mM pyruvic acid, 0.5 μ g/ml transferrin, 0.5 ng/ml selenium, 10 μ g/ml insulin, 1 ng/ml EGF and 1 U of each gonadotrophin for long duration. After 8 days culture in 4-well, we could observe typical follicle-like structure morphology, and this characteristics were continued for 26 days (14 ~ 40 days from the beginning). When gene-expression related germ cell and oocyte differentiation was examined using RT-PCR analysis, Vasa (a marker of postmigratory germ cells), FIG- α (a



transcription factor required for the expression of ZP protein) and three zona pellucida (ZP1, ZP2 and ZP3) were all expressed in those follicle-like structures. Also, after 35 days culture, we confirmed ZP protein expression in some of oocyte-like structures using immunocytochemistry. Further study on developmental potential as in vitro fertilization and blastocyst development in those developed oocyte-like structures is under way. These results concluded that oocyte-like structures can be derived from the mouse embryonic stem cells in vitro.

Keywords: *Embryonic stem cell, Oogenesis, In vitro differentiation*