fertilized and cultured in a 50 μl drop overlayed with WMO or SPO. The in vitro development rates were then examined and compared. Also, day 2 embryos grown in vitro under WMO overlaying were cultured in the two types of oil (WMO and SPO) or in the oil plus co-culture (WMO + CC and SPO + CC) using adult ear skin fibroblasts for 6 days. Cell number and hatching development rates were examined in developed blastocysts in each treatment group during subsequent culture for 48 hr.

Results: WMO or SPO overlaying resulted in significantly different bovine follicular oocyte development from day 6 embryo development after IVF (morula: 30.6 vs. 44.8%, blastocyst: 21.7 vs 32.8%, respectively) ($p<0.05$). Also, treatment of the day 2 embryo cultures with SPO overlaying or oil plus CC (WMO + CC or SPO + CC groups) reached significantly higher development rates from the morula stage compared to embryo cultures treated with the WMO overlaying ($p<0.05$). However, the development rates of the SPO treatment group (morula: 72.7, blastocyst: 53.1%) were slightly high compared to development of the culture treated with WMO + CC (69.6%, 50.4%, respectively).

Conclusions: This similar developmental competence pattern was also observed in cell number and embryo hatching rate. Therefore, SPO overlaying alone can support similar developmental competence as WMO overlaying plus co-culture for bovine embryo development in vitro. Thus, the oil selection for culture significantly influences pre-implantation embryo development in vitro.

P-4 Derivation of Oocyte-like Structure from Mouse Embryonic Stem Cells

Hwang Yun Cho[1], Kil Soo Jeon[1], Eun Young Kim[1,2], Won Don Lee[2], Se Pill Park[1,2], Jin Ho Lim[2]

[1]Maria Infertility Hospital Medical Institute/Maria Biotech, [2]Maria Infertility Hospital

Background & Objectives: 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.

Method: To differentiate in vitro into oocytes, three types of ES cells were plated in tissue culture plate at a density of $1\sim2.5 \times 10^4$ cells/cm^2 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- supplemented with 0.3% BSA, 0.23 mM pyruvic acid, 0.5 μg/ml transferrin, 0.5 ng/ml selenium, 10 μg/ml insulin, 1 ng/ml EGF and 1 U of each gonadotrophin for long duration.

Results: 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- (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.

Conclusions: 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.

P-5 Half-ICSI: An Insemination Method to Prevent First Cycle Fertilization Failure or Low Fertilization In Non-male Factor Infertility

Choi DH[1,2], Park LS[1,2], Kwon H[1,2], Choi MA[1], Kang KY[1], Kim EK[1], Chung HM[2], Cha KY[1,2]

[1]Infertility Medical Center of Pundang CHA General Hospital, [2]Pochon CHA University, Korea

Background & Objectives: To determine an optimal insemination technique in patients undergoing first IVF trial.

Method: Between January 2001 and July 2005, a total of 247 patients undergoing their first IVF cycle due to female factor or unexplained infertility, were included in the study and in which Half-ICSI were performed. 3580 oocytes were retrieved and 3082 sibling oocytes were randomly allocated to conventional insemination (1564 oocytes) or ICSI (1518 oocytes). The rates of fertilization and cleavage were compared in two groups. Patients with poor responder. Ovum donation and male factor patients were excluded.

Results: The mean age (M±S.D) of the patients was 32.8±4.0 years old, and the duration of infertility was 5.2±3.1 years. The mean number of oocytes retrieved per patient was 14.5±7.4. The fertilization rates following conventional insemination and ICSI were 77.2% (1207/1564) and 84.9% (1289/1518) respectively in total studied patients. Fertilization rate after ICSI was significantly higher than that after conventional insemination in non-male factor infertility patients. Complete fertilization failure occurred in two unexplained infertility patients following conventional insemination.

Conclusions: ICSI could be used to solve the unexpected fertilization problem such as reduced or absent fertilization in couples with unexplained infertility or other female factor infertility. A trial of Half-ICSI in the first cycle of IVF could be considered to reduce fertilization failure or low fertilization rate, especially, in couples with non-male factor infertility.