

P-2 Abdominal Ultrasound-guided Embryo Transfer Improves Clinical Pregnancy Rates in Repeated IVF-ET Failure Patients

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Background & Objectives: To compare the effectiveness of abdominal ultrasound guided embryo transfer with traditional method (embryo transfer by clinician's feeling) in repeated IVF-ET failure patients.

Method: From March 2003 to July 2005, 107 patients who underwent IVF-ET after at least 3 unsuccessful ART cycles were retrospectively evaluated. Group I, Ultrasound guided ET (n=54) and Group II, ET with traditional method (n=53). Transfers of cryopreserved embryos and fresh embryos from oocyte donation were not included in the study. ET was performed by the same physician.

Results: There were no differences between two groups regarding age (34.6 ± 4.2 ; 35.0 ± 4.5), infertility durations (6.6 ± 3.9 ; 6.9 ± 3.5), and cycle numbers (4.3 ± 0.8 ; 4.1 ± 0.8). Although not statistically different, the clinical pregnancy rate of the US-guided ET [53.7% (29/54)] were higher than that of traditional ET [39.6% (21/53)]. Implantation rate of the US-guided ET [18.6% (42/226)] was significantly higher than that of traditional ET [11.2% (31/276)].

Conclusions: Abdominal ultrasound-guided ET can increase IVF success rate in patients who had previously failed to conceive with traditional embryo transfer (ET according to clinician's feeling). The ultrasound-guided embryo transfer can be recommended as a routine embryo transfer procedure in the repeated IVF failure patients.

P-3 Sterile Filtered Paraffin Oil Supports In Vitro Developmental Competence in Bovine Embryos Comparable to Co-culture

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Background & Objectives: The most commonly used culture environment has been the micro drop culture method using oil rather than large scale medium, with the exception of certain specific culture methods. This study was to investigate whether sterile filtered light paraffin oil (SPO) overlaying supports in vitro developmental competence of bovine follicular oocytes better than washed light mineral oil (WMO) overlaying. In addition, the effects of the two types of oil overlaying were compared with oil overlaying plus co-culture (CC) on bovine embryo development in vitro.

Method: Bovine follicular oocytes were retrieved from a slaughtered ovary, matured in vitro, and then

fertilized and cultured in a 50 µl drop overlaid 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

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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 $1 \sim 2.5 \times 10^4$ 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- 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