

aneuploid. Furthermore, the aneuploidy rate was higher in Mid and Low quality blastocysts. Therefore, FISH results are necessary for adequate selection of euploid embryos for transfer.

Supported by: None.

PROCEDURES AND TECHNIQUES-LABORATORY: ART

Tuesday, October 18, 2005
3:45 p.m.

O-246

Oxidative Stress Induced Alterations in the Mouse Oocyte Cytoskeleton. W. Choi, R. K. Sharma, A. Agarwal, W. Paik, T. Falcone. Cleveland Clinic Foundation, Cleveland, OH; Gyeongsang National University, Jinju, Republic of Korea.

OBJECTIVE: Oxidative stress may induce alterations in the cytoskeleton of the oocytes. Damage to the cytoskeleton in mature oocyte, especially the spindle, which is comprised of microtubules, can result in failure of the final meiotic reduction division. We examined the alterations in the cytoskeleton and chromosome alignment of the metaphase II (MII) oocytes under conditions of oxidative stress induced in vitro.

DESIGN: Prospective, experimental animal study

MATERIALS AND METHODS: Mature metaphase II mouse oocytes (frozen) were exposed to various concentrations of hydrogen peroxide (H_2O_2): 12.5, 25, 50, or 200 μM . Another set of oocytes was exposed to 25 μM H_2O_2 for varying incubation times (15, 30, 45 and 60 min). Immunohistochemical staining was used to evaluate the effect on oocyte microtubule morphology and chromosomal alignment. Fixed oocytes were incubated with anti- α -tubulin monoclonal antibody for microtubule staining, followed by incubation with FITC labeled anti-mouse IgG antibody. For chromosome staining, oocytes were incubated with propidium iodide. Stained oocytes were scored for alterations in microtubule morphology and chromosomal alignment under a Fluorescent (Leica, Germany) and scanning Confocal microscope (Leica Lasertechnik GmbH, Heidelberg, Germany). Scores of 1-2 were considered as being normal for oocyte microtubule morphology and chromosomal alignment, and 3-4 as abnormal (modified from Saunders and Parks, 1999).

RESULTS: Compared to controls (Fig. 1A) H_2O_2 concentration significantly affected both spindle morphology and chromosomal alignment. Significantly higher scores were seen for both microtubule and chromosome alignment indicative of oxidative stress induced damage with >25 μM H_2O_2 . This increase in the damaging effect was dose dependent. In addition the effect of 25 μM H_2O_2 on alterations in both microtubule and chromosome alignment was significantly increased with increasing period of incubation. Significant damage in microtubule and chromosome alignment was observed in first 15 mins of exposure to oxidative stress.

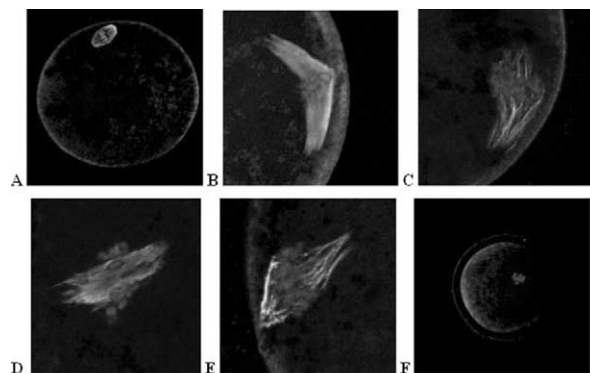


Fig. 1. Confocal microscopic photomicrographs of microtubule and chromosome in mouse oocyte (metaphase II) (A): showing normal characteristic barrel shaped spindle structure, (B-C) damaging effect of 25 and 50 μM H_2O_2 exposure for 30 min showing alterations in microtubule and chromosome alignment, and (D-F): changes following 25 μM H_2O_2 exposure for 30, 45 and 60 min.

CONCLUSION: Oxidative stress leads to disruption of the MII spindle in mouse oocyte in dose and time dependent manner. This is important while handling oocytes in vitro. Reducing the exposure time during assisted reproductive techniques may help minimize oxidative stress and improve the quality of the oocytes.

Supported by: None

Tuesday, October 18, 2005
4:00 p.m.

O-247

A Feeder Cell- and Serum-Free System To Harvest Embryonic Stem Cells From Laser Dissected Blastocysts. T. Takeuchi, N. Tanaka, Q. V. Neri, Z. Rosenwaks, G. D. Palermo. Cornell University, New York, NY.

OBJECTIVE: The key role of mouse embryonic fibroblasts (MEF) is recognized as the feeder cell layer for establishing ESCs. However, feeder cell culture is technically involving and more importantly is a source of contamination with xenogenic by-products. Recently, it has been identified that human ESCs are coated with immunogenic sialic acid. Moreover, the adoption of methods aimed at directly isolating inner cell masses (ICM) by immunosurgery, entails exposure to allogenic antibodies and complement. Therefore, the avoidance of feeder cells and serum-free media as well as the utilization of mechanical ICM dissection would be ideal. In this study, we attempted mouse ESC derivation without feeder cells and sera. To facilitate ICM isolation, laser-assisted blastocyst dissection was performed.

DESIGN: In order to assess the efficiency of ESC harvesting in different culture systems, laser dissected blastocysts were plated either on a feeder cell layer or directly on gelatin-coated culture dishes. Cells were harvested, cultured, and maintained in a medium supplemented with a defined protein cocktail. Intact blastocysts on MEF were used as control.

MATERIALS AND METHODS: Zygotes were obtained from mated 7-11 week old $B_6D_2-F_1$ mice, and cultered to the blastocyst stage. Laser-assisted ICM isolation was performed on blastocysts on embryonic day 3. An intact blastocyst was secured by two holding pipettes with the ICM being positioned at 9 o'clock. Once adequate tension was established, several (8-10) infrared laser pulses (300 mW, 1 μs) were fired to section the blastocyst into two uneven portions, one contains the ICM while the other only trophoblastic cells. The isolated ICM components were randomly plated either on MEF feeder layer or directly on gelatin-coated dishes, and cultured in a chemically defined medium supplemented with serum substitute and LIF (2000 IU/ml). Intact blastocysts on MEF were used as control. The ICM growth was monitored daily by size measurements. First dissociation was carried out only when at least 100 μm diameter of the plated ICM was reached, without endodermal differentiation. ESC colonies so derived were propagated, fixed, and process for alkaline phosphatase, Oct-4, and TROMA-1 activity.

RESULTS: A total of 21 intact blastocysts were laser dissected and 10 of them were plated on MEF while the remainder ($n = 11$) on gelatin-coated dishes. Laser dissection was successful in all cases. In presence of MEF, attachment occurred at a similar rate than in their absence (90% vs 82, respectively). ICM growth patterns were similar among the two culture methods. A total of 3 (30.0%) ESC lines were established on feeder layers while 1 (9.1%) on gelatin. Intact blastocysts ($n = 73$) cultured in a standard fashion (with feeder cells) yielded 20.5% of colonies. The cell lines obtained with laser dissection, whether on feeder cells or not, displayed the same quality and grade, as judged by morphological and pluripotency markers, of ESC lines obtained from intact blastocysts.

CONCLUSION: With laser-assisted ICM dissection, we were able to obtain in standard culture conditions, supported by feeder cells, ESC colonies at a similar rate than intact blastocysts. With the same method, we were able to generate an ESC line in feeder cell- and serum-free culture system. This preliminary data represents a feasible model to establish ESC lines in a controlled and xenogenic by-product free environment.

Supported by: Institutional