UNIVERSITI TEKNOLOGI MARA

THE DOCTORAL RESEARCH ABSTRACTS

Volume: 11, Issue 11 April 2017

ELEVENTH ISSUE

INSTITUTE of GRADUATE STUDIES

IGS Biannual Publication

49

Name : WAN HAFIZAH BINTI W. JUSOF

Title : RELATIONSHIP BETWEEN TIMING OF THE FIRST ZYGOTIC CLEAVAGE WITH CYTOSKELETAL STRUCTURES AND AMINO ACID METABOLIC PROFILES IN VITRIFIED MOUSE EMBRYOS

Supervisor : PROF. DR. NOR ASHIKIN MOHAMED NOOR KHAN **(MS)**
PROF. DR. MOHD HAMIM RAJIKIN **(CS)**
ASSOC. PROF. DR. NURALIZA ABDUL SATAR **(CS)**

Timing of the first zygotic cleavage has been used as a marker of embryo developmental competence and subsequent viability. Previous studies showed that embryos that cleaved early had higher developmental viability. However, the factors contributing to timing of the first zygotic cleavage are unknown. Energy production from mitochondria, nucleus and cytoskeletal organization might be some of the factors involved. Amino acid metabolic profiles might also relate with timing of the first zygotic cleavage as it has been reported to have significant relationships with embryo viability. Thus, the present study was designed to investigate the relationship between timing of the first zygotic cleavage, amino acid metabolic profiles, mitochondria, nucleus and cytoskeletal organization of mouse embryos with subsequent viability. Embryos were retrieved from superovulated ICR mice, 28 hours after hCG injection. At this point of time, 2-cell stage embryos were categorized as early-cleaving (EC), while zygotes with two pronuclei as late-cleaving (LC) embryos. Embryos were cultured overnight in M16 medium supplemented with 3% Bovine Serum Albumin (BSA) in a humidified carbon dioxide (CO_2) incubator. For Experiment 1, both EC and LC embryos were divided into control and treatment groups. For control group, 2-cell stage embryos were cultured until the blastocyst stage. For treatment group, embryos were vitrified by EFS40 or EFS20/40 method for 1 hour and warmed. The vitrified-warmed embryos were cultured until the blastocyst stage. The number of surviving embryos and their development to the blastocyst were observed and counted. For Experiment 2, 2-cell stage embryos were divided into control (non-vitrified) and treatment (vitrified) groups. Embryos in both control and treatment groups were fixed in 4% paraformaldehyde and immunostained to visualize the localization and intensities of mitochondria, actin, tubulin and nucleus. Finally, the embryos were mounted on slides and examined under a Confocal Laser Scanning Microscope. The structures intensity were analyzed by LAS-AF-Lite Software. For Experiment 3, EC and LC embryos were cultured individually in 4 µl drops of KSOM/AA medium supplemented with 3% BSA. The embryos were transferred every 24 hours to fresh 4 µl drops of KSOM/AA + 3% BSA until the blastocyst stage. The spent culture medium was analyzed by Ultra Performance Liquid Chromatography (UPLC) for amino acids metabolic profile. Results showed that nonvitrified and vitrified EC embryos had significantly higher developmental viability and higher cryosurvivability after vitrification by EFS40 and EFS20/40 method. Confocal analysis showed that non-vitrified and vitrified EC embryos had a significantly higher densities of mitochondria, actin and nuclear chromatin compared with non-vitrified and vitrified LC embryos, which appear to result in more efficient cell division, and therefore greater developmental competence. Amino acids metabolic profile showed that EC embryos had a significantly lower amino acids turnover compared to LC embryos in both Day-2 to Day-3 and Day-3 to Day-4 of cultures. These findings suggest that the higher developmental viability of EC embryos was significantly attributed by lower metabolic activity. In conclusion, timing of the first zygotic cleavage was associated with mitochondria, nucleus and cytoskeletal ultrastructure and amino acids metabolic profile that affect subsequent developmental viability and cryosurvival of embryos.