

MATERIALS AND METHODS: The first and second meiotic divisions were studied using fluorescent and time-lapse microscopy. Controlled compression of oocytes prevented rotation of the first meiotic spindle, which remained parallel to the surface of the oolemma. This resulted in the division of the maturing oocytes into two approximately equal cells, one of which was equivalent to an enlarged first polar body. Such 2-celled oocytes were cultured with sperm after localized openings were made in their zona pellucida using laser. Fertilization and embryonic development were photographed at frequent intervals.

RESULTS: Each of the two cells in the oocytes established a second meiotic spindle. Both cells were fertilizable, released a second polar body and formed a male and female pronucleus. Each cell cleaved to establish twin 2-cell embryos within the zona pellucida. After each twin embryo had cleaved to beyond the 4-cell stage, it became difficult to distinguish individual embryos due to mingling of their blastomeres. Following intimate aggregation of blastomeres a single compacted morula was established. This progressed to a blastocyst, but expansion failed to occur. In some cases, both cells were fertilized within the zona but only one produced a cleaving embryo. In such cases the developing half produced a blastocyst that contained a large neighbouring cell.

CONCLUSIONS: A single oocyte may give rise to twin intra-zonal embryos that subsequently combine to form a chimera, or a mosaic embryo, containing two maternal and two paternal genetic contributions. It can be deduced that the composite embryo could contain either an XX/XX, or XX/XY, or XY/XY genotype. But the developmental potential of such chimeric entities needs to be evaluated using genetic analysis. In addition, if the original twin embryos do not combine, their separate development with fusion and sharing of trophoblasts, could give rise to monochorionic twins.

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3:30 pm

O-126

MEIOTIC SPINDLE LOCALIZATION AND MICROFILAMENT DISTRIBUTION DURING OOCYTE VITRIFICATION. C.-C. Chang, Z. P. Nagy, L.-Y. Sung, H. I. Kort, X. Yang, C. X. Tian. Reproductive Biology Associates, Atlanta, GA; University of Connecticut, Storrs.

OBJECTIVE: During female meiosis, most of the maternal resources are retained in the oocyte for the early developing embryo. This outcome is coordinated by asymmetrical divisions. Two significant events ensure these asymmetrical divisions occur during meiosis: first, the meiotic spindle migrates towards the cell cortex, and second, a microfilament-rich cortical domain over the spindle is formed. It is not known if cryopreservation may impact these delicate arrangements which determine these critical processes. Our objective, therefore, was to investigate the specific changes of oocyte spindle localization and microfilament distribution before, during, and after vitrification.

DESIGN: Timed observations were performed before, during, and after vitrification.

MATERIALS AND METHODS: Mature oocytes were collected from 8-wk-old BDF1 mice with standard superovulation. Cryopreservation on all oocytes was performed by the vitrification method with 15% ethylene glycol, 15% DMSO and 0.5 M sucrose. Oocyte warming was performed after vitrification by serial dilutions in three steps using 1.0M, 0.5M, and 0M sucrose solutions. To examine the spindle, the oocytes were fixed at 5 min of equilibration solution, 1 min of vitrification solution, 0 min of warming (warming directly into fixative), and 1h after warming, and stained with propidium iodide and FITC-conjugated anti-tubulin antibody by confocal microscopy.

RESULTS: A total of 75 oocytes were included in the present study. After 5 min exposure to equilibration solution, all of the meiotic spindles were positioned at the microfilament-rich domain of the oocyte's cortex (16/16) as the control group before exposure to cryoprotectant (12/12). All oocyte spindles were still localized to microfilament-rich domain of the oocyte's cortex in vitrification solution (15/15) with even higher concentration of cryoprotectant and the subsequent cooling process (15/15). We observed that all examined oocytes sustained consistent microtubule and microfilament structures at 1h after warming (17/17).

CONCLUSIONS: This study demonstrated that the spindle localization and microfilament distribution were well conserved throughout the cooling and warming processes, even though oocytes were exposed to high concentrations of cryoprotectants and non-physiological temperatures for substantial time. This study thus indicates that oocyte cryopreservation using vitrification may be a suitable technique in regard to maintaining cytoskeletal structure.

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O-127

EMBRYONIC STEM CELL (ESC) PLURIPOTENCY REGULATORS HAVE NOVEL AND CRITICAL ROLES IN PRE-BLASTOCYST DEVELOPMENT. K. M. Foygel, S. H. Jun, D. E. Leong, B. Choi, W. H. Wong, M. W. M. Yao. Ob/Gyn, Stanford University School of Medicine, Stanford, CA; Applied Physics, Stanford University, Stanford, CA; Statistics, Stanford University, Stanford, CA.

OBJECTIVE: Gene regulation and reprogramming during the maternal-embryonic transition in the early embryo have remained elusive despite our understanding of the ESC gene network. Based on their transcript abundance, we hypothesized that the ESC pluripotency regulators, *Sall4*, *Oct4*, and *Sox2*, may be required for pre-blastocyst development, and their precise functions would help us to understand mechanisms controlling early embryo development.

DESIGN: Gene-specific, antisense morpholino oligonucleotides (MOs) were injected into the cytoplasm at the 2PN stage to block translation and induce gene knockdown (KD) of each of *Sall4/Oct4*, and *Sox2*, via steric hindrance. Maternal and embryonic transcripts are thus simultaneously targeted in a way that is not possible by conventional knockout mouse models.

MATERIALS AND METHODS: We compared the developmental effect of each gene KD to its uninjected and mismatch controls in ≥ 3 independent experiments by Student's t-test. Protein expression was tested by immunocytochemistry. Global gene expression profiles were obtained from KD and control embryos at the 2-cell stage as per protocol (PicoPure[®] kit, Molecular Devices; Pico-ovation[®], Nugen; GeneChip[®] Mouse Genome 430 2.0 Array, Affymetrix).

RESULTS: We found that *Sall4*, *Oct4*, and *Sox2* were each critical for early embryo development. Specifically, *Sall4*, *Oct4*, and *Sox2* were required for development beyond the 4-cell, multicell, and morula stages, respectively. Few embryos reached the blastocyst stage, if at all. Further, successful translational block was confirmed by abrogation of nuclear signals. Finally, analyses of global expression profiles of *Sall4* and *Oct4* KD models suggest novel roles in cell cycle regulation, apoptosis, chromatin remodeling and epigenetic regulation, via transcriptional and posttranscriptional mechanisms.

TABLE 1. Developmental Effects of Gene Knockdown

Gene Knockdown	Stage of Arrest	% Arrest MO	% Arrest Mismatch	% Arrest Uninjected	p-value (MO vs mismatch)
<i>Sall4</i>	1- to 4-cell	76.2 ± 10.9	4.2 ± 4.2	6.7 ± 6.7	0.013
<i>Oct4</i>	1- to multicell	89.6 ± 5.8	18.9 ± 8.1	0	0.003
<i>Sox2</i>	multicell, morula	78.5 ± 11.5	5.6 ± 2.9	0	0.019

CONCLUSIONS: By combining MO-mediated gene KD and global gene expression profiling, we discovered that *Sall4*, *Oct4*, and *Sox2*, have novel and critical roles in normal embryo development. More broadly, we propose to apply our strategy to dissect the dynamic gene network that directs early embryo development.

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4:00 pm

O-128

VITRIFICATION OF HUMAN EMBRYONIC STEM CELLS WITH CRYOVIALS. T. Li, C. Zhou, Q. Mai, Y. Shu. Reproductive Med Ctr, Department of OB&GYN, The 1st Affiliated Hospital, Sun Yat-sen University, Guangzhou, China; Reproductive Med Ctr, Department of OB&GYN, The 1st Affiliated Hospital, Sun Yat-sen University, Guangzhou, China; Reproductive Medical Ctr, Department of OB&GYN, The 1st Affiliated Hospital, Sun Yat-sen University, Guangzhou, China; Reproductive Med Ctr, Department of OB&GYN, The 1st Affiliated Hospital, Sun Yat-sen University, Guangzhou, China.

OBJECTIVE: The traditionally used vitrification method is no longer suitable for the vigorously proliferating human embryonic stem cells (HES) because of mini vitrification carriers. Recently we have reported a bulk vitrification (BV) method with cell strainer, which could cryopreserve a large