Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

Innovation and technology

Magnetic 3D-cell culture as a new system to generate spheroid derived from oviductal cells with a wide range of applications

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Resumo

The oviduct plays roles in gametes and embryo transport, sperm capacitation, fertilization, and early embryo development. Even though some in vitro models for culturing oviductal cells are available, yet, there is no gold standard system. Some limitations are cellular dedifferentiation, limited cell lifespan, and/or complex methodologies. Therefore, our aim was to develop a new effective system to generate spheroid derived from oviductal cells. For this, we use the magnetic 3D-cell culture system (Greiner Bio-One CELLSTAR®, Germany), which is widely used for many cell types, and, to our best knowledge, has not yet been tested on oviduct cells. Bovine oviduct epithelial and stromal cells, collected from an abattoir, were separately cultured in a monolayer system. At 80% confluence, cells were trypsinized, counted, and magnetized by centrifugation with the nanoshuttle™-PL (NS). Next, cells were seeded in 96-well plates with a cell-repellent surface and placed atop a magnetic plate. The magnetic forces aggregate the cells to form a spheroid. First of all, cells were seeded as 50,000, 25,000, 10,000, and 5,000 cells/well. Within three days of culture, both epithelial and stromal cells were able to aggregate forming 3D structures of attached cells denominated as Oviductal Magnetic Spheroid (OMS). Regarding their size, 50,000 and 25,000 cells were oversized, resulting in a necrotic center of propidium iodide positive cells due to restriction of nutrients access, whereas the 10,000 and 5,000 cells sizes were capable of keeping cells alive in the spheroid. Therefore, the next experiments were performed with 10,000 cells per OMS. Afterward, we tested: 1) proportion of the NS per cell (0.5, 1.0, and 1.5 µL NS/104 cells), 2) ratio of epithelial to stromal cells (9:1, 7:3, and 5:5, respectively), and 3) OMS formation in 1-step (epithelial and stromal cells seeded together) or 2-steps (epithelial cells seeded 24h after stromal cells). Taking into consideration the reproducibility of the spheroid formation, the number of non-attached, and cell survival, it was designated as most fitting the proportion of 1.0 µL NS/104 epithelial cells, 0.5 µL NS/104 stromal cells, the ratio of 7:3, and no difference between 1- and 2-steps for cell seeding. Last, after 7 days of culture, we observed that our model has the capacity for self-organization, stromal cells (anti-vimentin positive) and epithelial cells (anti-cytokeratin positive) rich in primary cilia (scanner electronic microscopy) were situated, respectively, in the inner and peripheral area of the OMS, approximating to tissue architecture. Altogether, these data show a strong possibility of using the magnetic system to perform a new in vitro culture system for oviductal cells, which is tempting to hypothesize that this model will be useful to evaluate oviductal cell redifferentiation, response to hormone stimuli, embryo development, and maternal-embryonic cross talk. Supported by FAPESP (19/25982-7, 20/02500-4).

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Liquid marbles micro-bioreactor as a new system for in vitro bovine oocyte maturation and embryo production

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Resumo

Liquid Marble (LM) is a small drop of liquid encapsulated by hydrophobic powder particles allowing cells to be cultivated in a three-dimensional manner in a small liquid system. This study aimed to evaluate the use of LM during cumulus-oocyte complexes (COCs) in vitro maturation (IVM) and embryo in vitro culture (IVC) system. For the IVM experiment, COCs aspirated from slaughterhouse ovaries were divided into two different groups for IVM: 1) control (bi-dimensional culture using conventional drops) and; 2) LM group. Seven replicates were performed with n=20 oocytes per replicate per group. After 21 hours of IVM, the COCs were denuded to remove cumulus cells (CC) and the IVM-rates were evaluated by the second polar body extrusion. CC and denuded oocytes were collected, snap-frozen, and stored at -80ºC for expression analysis. For the IVC experiment, COCs from slaughterhouse ovaries were aspirated, selected, conventionally in vitro matured, and fertilized. After 18 hours of in vitro fertilization, 120 presumptive zygotes per replicate, were denuded to remove CC and divided into two groups for IVC: 1) control; and 2) LM group. For IVC experiment, eight replicates were performed and 3 pools of 5 blastocysts (each pool containing 2 blastocysts and 3 expanded blastocyst) per group were obtained on Day 7 for further 380 miRNA profile assessment, which are responsible for regulating ~60% of bovine genes. RNA was extracted using QIAzol, converted in cDNA and qPCR for the genes EIF4b, EIF4e, BAX, BCL2, CDK6, HAS2, GAPDH, and FOXO3a in CC and GDF9, BMP15, PI3K, PTEN, FOXO3a, BAX, and BCL2 in oocytes. Cycle threshold for each gene were normalized by the geometric mean of PPIA and ACTB. MiRNA reverse transcription was performed using miScript II RT Kit (HiSpec) in Day 7 blastocysts. Relative expression bovine miRNAs were determined by RT-qPCR with SYBR Green PCR kit (QIAGEN) and the miR-99b was used as housekeeping. Statistical analyses were performed by Student's t-test, a $P<0.05$ was considered for statistical difference. We found that the IVM rate did not differ between groups ($P=0.5633$). In CC, EIF4e (involved in the initiation of gene translation in eukaryotes), BCL2 (encodes an anti-apoptotic protein) and GAPDH (enzyme that breaks down glucose for energy and carbon molecules) had their expression reduced in LM compared with control. In IVC experiment, a total of 32 miRNAs were identified, and the miRNA miR-615 was downregulated in the LM, and the bta-let-7f was exclusively expressed in the control. These results suggested that a three-dimensional culture based on LM was capable of modulate the cumulus cells and the blastocysts at molecular levels. However, more analyses investigating the use of LM on IVM and IVC are necessary to generate a solid knowledge of the biological pathways affected in CC and blastocysts.

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A PROSPECTIVE THREE-DIMENSIONAL MICROENVIRONMENT DERIVED FROM PLACENTAL MICE ECM

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Resumo

Human placenta physiology is usually described using samples derived from human term and unsuccessful pregnancies, or from mice model. Which means that human placental physiology is not completely elucidated. Furthermore, new proposals are necessary to reconstruct placental fragments, by bioengineering strategies, given support to simulate placental physiology. Then, herein we aimed to valited by protein content the decellularized mice placental scaffold as a microenvironment able support and maintain cell culture. For this, control (n=3) and SDS-decellularized (n=3) 18.5-day old mice placenta were grouped by condition and washed, lysed, urea-reduced, acetone-precipitated, DTT-reduced, iodoacetamide-alkylated, trypsin digested, and C-18 column purified. At the end, 3 µg protein were loaded in Orbitrap Fusion Lumos spectrometer (ThermoScientific). Generated spectra were exported to MaxQuant software (v1.6.10.43) to produce the protein list of each sample, and the LFQ intensity were statistically analyzed by Inferno software (v.1.1.6970). A list of 2,317 proteins were detected and 118 (5.1%) proteins were filtered using extracellualr matrix (ECM) and cell junction-related ontologies. Control and decellularized conditions equally regulated 76 (64.4%) ECM (collagens, laminins, fibrillin, fibronectin, glycoproteins) and cell junction-related proteins. The enriched ontologies in cellular component domain were related to cell junction, collagen and lipoprotein particles; whereas in biological process domain, we found cell adhesion, vasculature, proteolysis and ECM organization; while in molecular function there were protein binding and activity and ECM resistance ontologies. From the enriched pathways, we could cluster them in cell adhesion and invasion, and labyrinthine vasculature regulation for placental nutrition. Furthermore, trophoblast cells do not survive for long periods in bidimensional in vitro culture models, then mimetize the adequate tridimensional placental microenvironment is critical to allow materno-fetal barrier assays. Finally, the maintenance of several collagen types associated with other fibrous and adhesive proteins on decellularized placenta can support the preservation of a stable tridimensional architecture shiftiness.

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