Animal Reproduction

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Innovation and technology

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

Patricia Kubo Fontes [1], Heloise Cale da Rocha [1], João Vitor Alcantara da Silva [1], Aldcejam Martins da Fonseca Júnior [1], Jessica Ispada [1], Marcella Pecora Milazzotto [1]

[1] UFABC - Federal University of ABC (Laboratory of Embryonic Metabolism and Epigenetic (LEME), Center of Natural and Human Science, Santo André, São Paulo, Brazil)

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).