Animal Reproduction

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Embriology, developmental biology and physiology of reproduction

Establishment of a tridimensional (3D) organoids culture using bovine endometrial glandular epithelial cells

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Resumo

In ruminants, the pregnancy begins with the migration of the blastocyst into the uterus. At this stage, the uterus supports the early embryo development up to the embryo implantation. The endometrium is composed by different cell types, including epithelial luminal, epithelial glandular and stromal cells. This cells play an important role in the uterine environment, improving receptivity to the embryo, implantation, and supporting conceptus elongation. Therefore, the main objective of this project is to establish the culture of tridimensional (3D) cell-derived bovine endometrial glandular epithelial cells to generate a model to study in vitro maternal embryo communication. The main hypothesis of the project is that the culture of organoids from glandular epithelial cells is feasible in cattle and may serve to study in vitro maternal embryonic communication. For the isolation of glandular epithelial cells, non-pregnant uteruses were selected in early luteal phase, based on the CL. The tissue dissection was performed in the Ipsi lateral horn and in the intercaruncular region. The tissue was incubated in 5mL of digestion solution (1mg/mL of collagenase) for 1 hour at 37°C, subsequently filtered through a 100 and 40 µm mesh and washed with 10% FBS in PBS Ca2++ and Mg2++ free and centrifuged at 600g/10min. The cellular precipitate was resuspended in 1mL of DMEM-F12 with 10% FBS and antibiotics (50µg/mL streptomycin and 50IU/mL penicillin), then they were seeded at 1 x 105 cells/mL in 75 cm2 culture flasks. After purifying the cell line, 3rd passage, the epithelial cells were cultured in 96-well plates with 5.000 cells/well in Matrigel drop for formation of 3D culture of epithelial cells. After 48 hours of culture, the cells were scraped to detach from the plate and went through two stes of centrifugations at 600g/10min, and thus replated again in 96-well plates with 5,000 cells/well. Three concentrations of Matrigel (5mg; 2.5 mg; 1.25mg) were tested. It was possible to observe the emergence of epithelial organoids after 96 hours, only at the 2.5mg concentration. Organoids showed an irregular shape at the beginning (96 hours) with a multicellular characteristic, and average area of 544.9±161 mm² and 81.4±13.9 mm of circumference. After 24 hours, the organoids became spherical, increased the area at greater diameter to 571.2±136.8mm with a lumen that was denser and frequently showed apparent cilia movement. The technique is still under development, as the project will contribute to the development of biobanks of bovine uterine organoids, to study the maternal embryonic communication and uterine receptivity within animal reproduction.

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CAPES.