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SPERM STORAGE TUBULES CULTURE: A NEW APPROACH FOR REPRODUCTIVE RESEARCH IN AVIAN SPECIES

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Sperm storage tubules (SST) are epithelial structures found in the mucosa of distal half of the oviduct of all avian species studied. These tubules maintain and store sperm up to 70 days and this allows fertilization without insemination. The aim of this work was to set up epithelial SST cells culture for future use as an *in vitro* model for oviduct cells-sperm interaction. Hens (*Gallus gallus domesticus,* Unité de Recherches Avicoles [URA], INRA, Nouzilly.) were euthanized with sodium pentobarbital injection. Oviducts were isolated and removed and the uterovaginal villi was manually dissected under stereomicroscopy. The SST area on the top of isolated mucosal villi was dissected, scalped in small fragments, and enzymatically digested in 1µg/ml Collagenase for 10 min at 41ºC. The digested tissue was flushed for 30 times by pipetting. The enzymatic activity was blocked by washing the tissue twice with culture medium. A second enzymatic digestion was performed by incubating the tissue overnight at 4ºC in 1µg/ml Pronase. The tissue was flushed again and the enzymatic activity was blocked. SST were isolated in 2 / 4% Percoll density gradient centrifugation at 2000g for 30 min at 4ºC. An intermediate phase of Percoll column containing SST was harvested before being maintained in Medium 199 containing 10% BFS and Gentamicin, during 30 min at 41ºC for fibroblast attachment. The medium containing SST was distributed in Lab-Tek Chamber Slide System (Nunc). SST were cultured at 37ºC, 5% CO_2 atmosphere, for 6 days. Immunocytochemistry for epithelial cell type confirmation, was performed with overnight incubation with monoclonal primary antibodies anti-Pan-cytokeratin (1:300, Sigma), Tubulin (1:300, Sigma) and Vimentin (1:500, Sigma) and anti-species secondary antibodies. We observed that, at the end of the enzymatic process, 90% dissected SST was isolated. In phase contrast microscopy we observed integral SST as well as individual cells. After 2 days of culture we observed cell migration from SST borders to form a monolayer. Eighty % cells presented epithelial characteristics as demonstrated with Cytokeratin and Tubulin positivity and Vimentin negativity, in Confocal microscopy. The digestion and isolation processes need to be controlled to differentiate the epithelial surface mucosal cells from SST cells. This method is very effective to isolate the SST specific population of cells that can be used in different reproductive and physiological studies for epithelial cell-sperm interaction.