Characterization of Three-dimensional Scaffold-based Model of the Porcine Endometrium *in Vitro*

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During the reproductive/estrous cycle, the endometrium of placental mammals undergoes cyclical remodeling. This process allows the endometrium to become receptive for the implantation of the developing embryo and prepare the uterus for pregnancy. Currently, the most popular research models are based on 2D *in vitro* cultures. However, it has been shown that culture on flat surfaces affects cells by changing their metabolism, gene expression and proliferation. Therefore, it has become important to develop solutions that will allow obtaining results as close as possible to *in vivo* conditions. One of the most promising solutions to achieve these goals is the use of 3D *in vitro* models. Three-dimensional endometrial *in vitro* systems may allow studying physiology aspects of the endometrium by reproducing the micro-environment where the early embryo develops. Therefore, the aim of the present study was to develop a 3D *in vitro* model of the porcine endometrium using synthetic polymer scaffold (Alvetex ®).

Endometrial stromal (ST) and luminal epithelial (LE) cells were enzymatically isolated from the uterus of gilts (n=3) in the mid-luteal phase of the estrous cycle. In the first stage, ST cells were seeded onto the Alvetex scaffold following guidelines provided by manufacturer and cultured for 3 weeks. During this time scaffolds were fixed at 3 time points (day 7, day 14 and day 21) for immunofluorescence and immunohistochemistry analysis. Extracellular matrix proteins (ECM) synthesis by ST cells was assessed and once an appropriate layer of ST cells was obtained, LE cells were seeded on the surface of the scaffold and cultured for another week (day 28) and fixed for analysis.

Hematoxylin-eosin staining cross-sections after 7, 14 and 21 days of culture showed that ST cells penetrated homogenously into the scaffold and showed typical fibroblast morphology. Moreover, ST cells showed positive vimentin staining throughout the culture period. Additionally, the presence of ECM was demonstrated on day 7, 14 and 21 of culture. After 21 days of culture, the stromal layer of endometrial fibroblasts served as a foundation to grow LE cells. On day 28 of culture, LE cells grew on the scaffold surface, showed positive staining with cytokeratin and typical epithelial morphology. The present report demonstrates that Alvetex scaffolds can support the porcine endometrial cell culture for long periods. Moreover, the reported approach is a promising model for the study of physiology of the endometrium and embryo-maternal interactions during early pregnancy.

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