Mucus Production in Conditionally Reprogrammed Endocervical Cell Cultures Derived from Human and Baboon Cytobrush Samples

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The endocervix regulates female fertility by producing cervical mucus that can permit or prevent sperm entry into the upper reproductive tract. We previously demonstrated that using conditionally reprogrammed primary endocervical cells from nonhuman primates (NHPs), we can study the regulation of cervical mucus secretion *in vitro*. However, our prior studies used primary cells harvested from necropsy specimens. Cell cultures produced from non-invasive cytobrush sampling would facilitate translation to human cell collection, allowing the study of unique genetic or disease states. Thus, we sought to determine if conditional reprogramming would also enable production of primary endocervical cell cultures of both NHPs and women, obtained non-invasively through the same endocervical cytobrush sampling technique used in cervical cancer screening.

We collected endocervical cytobrush samples from thirteen women, as well as six adult baboons (Papio hamadryas). We obtained cells by inserting a cervical cytology brush (CytoSoft, Fisher Scientific) into the endocervix and twirling the brush for approximately 3 seconds. Brush samples were enzymatically digested, cultured in conditionally reprogrammed conditions. Expanded cells were differentiated at an air-liquid interface, and subsequently treated with either vehicle control or estradiol for one week. We evaluated steroid hormone receptor expression, mucus production, and ultrastructural appearance of resultant cultures.

We successfully cultured primary cells obtained through cytology brush samples of four (67% success rate) baboon and four (36%) human endocervical cells. Using quantitative PCR, we confirmed the presence of both estradiol receptor (ER) and progesterone receptor (PGR) in our cytology brush generated cultures. Using WGA-lectin, a glycan specific carbohydrate binding protein that corresponds to the gel-forming mucin MUC5B, we found evidence of mucin production in all differentiated samples. Additionally, we compared our differentiated cell cultures to tissues using both TEM and SEM, demonstrating similar ultrastructural appearance.

Using conditional reprogramming, we successfully cultured primary endocervical epithelial cells derived from cytological brush samples of both NHP and women. Of note, this is the first study that has produced mucus secreting cell cultures from conditionally reprogrammed *human* endocervical cells. Endocervical cytology brushings are a non-invasive, universal, screening procedure routinely used with women. A technique to generate cell cultures from the scant starting material of brushings would bypass the need for animal or human organ donation. As

previous studies demonstrate that conditionally reprogrammed cells maintain genetic similarity to their parent tissues, this approach provides a possible experimental platform for individual-specific effects of drugs, toxins and pathogens on endocervical cells.