

Histological characterisation of the ovine cervical epithelium along the length of the cervix at different stages of the reproductive cycle

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Cervical artificial insemination (AI) for sheep using frozen-thawed sperm yield unacceptably low (<30%) pregnancy rates worldwide. A more thorough understanding of the ovine cervical environment is critical to develop strategies to improve sperm transport across the cervix. The aim of this study was to characterise the ovine cervical epithelium along the length of the cervix and to ascertain if it is altered by the stage of the reproductive cycle. Multiparous Texel cross Suffolk ewes were synchronised using a 12-day progestogen pessary followed by 400 IU of equine chorionic gonadotropin at pessary removal. The ewes were then slaughtered at either the follicular (Day 0; n = 6 ewes) or luteal (Day 10; n = 7 ewes) phase of their cycle following which their reproductive tracts were recovered and the cervix isolated. Circular segments (6 mm in length) were taken from each cervix along the entire length of the cervix (up to 10 segments per cervix), fixed using 10% formalin, wax-embedded, and stained with haematoxylin and eosin. Slides were digitally scanned and analysed using a combination of Imagescope, Qupath, and ImageJ (Fiji) software. The aim was to quantify the ratio of squamous vs columnar epithelial cells, assess the height of columnar epithelial cells and quantify the change in cervical fold surface area of the epithelium (measured as cervical fold linearity). All results are presented as mean \pm s.e.m. The cervical epithelium was predominantly comprised of columnar epithelial cells with squamous epithelial cells only found on the periphery of the first cervical segment at the external Os, mainly in the follicular phase. Columnar epithelial cells were exclusively found through segments 2 through 10 (1 = External Os; 10 = Internal Os) in both reproductive phases. The height of the columnar epithelium was greater in the follicular ($33.3 \pm 1.31 \mu\text{m}$) compared to the luteal ($26.7 \pm 1.21 \mu\text{m}$) phase ($P > 0.001$). There was a significant decrease in cervical fold linearity between the segments nearest the external Os and segments nearest the internal Os ($P < 0.01$). This was represented by the presence of significantly more crypts and folds in the segments nearest the internal Os. In conclusion, this study illustrates that the epithelium of the ovine cervix consists predominantly of columnar epithelium cells and these increase in height during the follicular phase in line with their mucus producing function. In addition, the increased folding of the cervical epithelium deeper in the cervix has implications for understanding the barriers to sperm transport across the cervix.