

Organoids as an *in vitro* pyometra model

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The canine endometrium is a hormonally responsive tissue composed of stromal fibroblasts and two epithelial cell types (luminal and glandular), which are organised into distinct crypt, intermediate, and basal zones (Barrau et al., 1975). Its structure and function are regulated by cyclical fluctuations in sex steroid hormones, particularly oestradiol and progesterone, during the oestrous cycle. These hormones act through specific nuclear receptors, whose expression patterns are dynamically regulated and may contribute to reproductive pathologies such as pyometra. Despite long-standing interest in the role of hormonal signalling in uterine disease, progress has been limited by the lack of physiologically relevant *in vitro* models.

Traditional 2D monolayer cultures fail to recapitulate the three-dimensional architecture and hormonal responsiveness of the endometrial tissue, while explant models lack long-term viability. Organoid technology provides a promising alternative (Thompson et al., 2020). Derived from tissue-specific epithelial cells, endometrial organoids offer a robust, hormone-responsive, and expandable 3D culture system that better mimics the native uterine environment. In this study, we established a protocol for isolating and cultivating primary endometrial epithelial cells from canine uteri and successfully developed long-term 3D endometrial organoid cultures. These organoids were then exposed to exogenous oestradiol and progesterone to evaluate receptor-mediated responses and cellular differentiation using histological and immunohistochemical methods.

Our goal is to utilise these organoids as an *in vitro* model to investigate hormone-receptor dynamics and their role in endometrial physiology and disease. Ultimately, this model could offer novel insights into the pathogenesis of hormone-associated uterine disorders such as pyometra, while also contributing to the reduction of animal use in research, in line with the 3Rs principles.

References

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