Comparative 3D Cell Culture Models of the Mammalian Endometrium to Understand the Evolution of Uterine Function

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Uterine function is critical to the survival of a species, but the exact mechanisms that ensure successful reproduction differ between mammals. Placental mammals innovated a cellular differentiation of the lining of the uterus called decidualization that prevents early parturition, such as that seen in marsupials. However, the initiation of decidualization differs across species. It can occur spontaneously, as in humans and menstruating primates, or in response to embryo implantation, as in most non-menstruating species. The endometrium, the mucosal layer of the uterus, is a key component of what defines a mammal, providing the interface between the mother and the fetus, but the extent to which its functions and structure are conserved have been underexplored. Establishing the similarities and differences in endometrial hormonal response across species will provide insight into mechanisms of uterine function that will lead to understanding of uterine dysfunction.

Endometrial tissue is dynamic and necessitates sampling at matched time-points to capture cycle-relevant functions such as decidualization. This synchronization of sampling is challenging and potentially confounded by inter-species differences in reproductive or life history traits. Moreover, collecting uterine tissues from multiple mammalian species requires invasive surgery or sacrifice of multiple individuals to appropriately document all time points in the cycle. In this context, cell culture models are a desirable alternative that allow for precise measurements at controlled cycle timepoints, and reduction of the number of animals used for research.

Recent 3D cell culture models of the human endometrium that incorporate both stromal and epithelial cell types have been developed, termed "assembloids". These models recapitulate many structural features of the endometrium *in vivo*, with epithelial gland-like structures embedded in a stromal matrix, that traditional cell culture models cannot reproduce. Inclusion of both cell types improves the model because stromal-epithelial crosstalk is required for structural changes across the cycle. Human endometrial assembloids can be treated with hormones in culture to induce decidualization. Extending the assembloid model to other mammals allows comparison of hormonal cycles across species with different cycle lengths because hormonal levels can be changed in a controlled manner.

To study the function and organization of the epithelial glands and stromal supporting cells, we developed *in vitro* 3D assembloid models incorporating epithelial organoids and stromal

cells across multiple species, including humans, mice, rabbits, and a menstruating species of bat (*Carollia perspicillata*). Here, we report the preliminary assessment of this new cross-species model to study the evolution of uterine functions. We observe qualitative differences in growth rates and internal structures of organoids across the different species. Further experimentation will allow comparative investigation of how changes to the structure and composition of the glands and tissue over time in response to various hormonal treatments will differ in species with spontaneous or induced decidualization.