



RESEARCH PROGRESS REPORT SUMMARY

Grant 03055: Evaluating Reproductive Diseases in vitro with a 3D Canine Endometrial Organoid Model

Principal Investigator: Fiona Hollinshead, BVSc, PhD
Research Institution: Colorado State University
Grant Amount: \$51,349.68
Start Date: 10/1/2022 **End Date:** 9/30/2024
Progress Report: Mid-Year 1
Report Due: 3/31/2023 **Report Received:** 6/21/2023

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Original Project Description:

Pyometra is a life-threatening disease that affects over 66% of intact older bitches. Bitches are predisposed to this disease due to a number of unique features of their reproductive cycle. Endometritis is an underdiagnosed condition in bitches that results in reduced fertility and can lead to the development of pyometra. These diseases have mostly been studied in live dogs resulting in welfare concerns and limitations in our understanding of the disease onset and progression and importantly, prevention of these conditions. Organoids are miniature organs in a culture dish that can be grown long-term while maintaining the characteristics and function of the original organ. This unique 3-dimensional (3D) structure facilitates the study of disease processes such as endometritis and pyometra in canines, potential treatments, and assessment of preventative therapeutics such as novel vaccines. Optimization of canine uterine organoids has the potential to: a) improve the health and welfare of intact middle-aged and older female dogs; b) enhance fertility, genetic gain and health in working dog programs; and c) allow the study of female reproductive diseases without the use of research dogs.

Publications:

No manuscripts have been submitted at this time, but a manuscript is in preparation that will be submitted upon completion of imaging and PCR for gene expression data, which are the final steps of Objective 1.



Presentations:

None at this time.

Report to Grant Sponsor from Investigator:

Three-dimensional (3D) organoid cell cultures present new opportunities to improve understanding of common reproductive pathologies in the bitch in a laboratory setting rather than using live animals for research. This is an improvement on using research dogs by: i) addressing important welfare and ethical concerns, ii) allowing more controlled study of cellular responses due to different hormones and infectious agents, and iii) permitting high-throughput evaluation of treatments performed in tandem. This 3D reproductive organoid cell culture technology has not been attempted in canines prior to these studies.

Thus far, we have demonstrated that organoids can be generated from canine endometrial tissue, which is the inner layer of the uterus. The tissues were collected from discarded reproductive tracts after a neutering surgery was performed at a local spay/neuter clinic. The organoids were grown for a total of 26 days in culture and maintained normal viable function and structure during this time. This long period of culture whilst maintaining physiologically viable cells is not possible using other traditional cell culture models. For the final 6 days in culture, organoids were exposed to hormonal treatments (i.e., estrogen and progesterone) to mimic what occurs during the reproductive cycle in the bitch.

We found that the canine endometrial organoids are structurally similar to endometrial organoids that have been developed in humans and horses. However, uniquely, canine endometrial organoids demonstrated color and morphology cellular changes in response to each hormone treatment. These changes mirror the normal physiological processes that occurs in the uterine lining during the reproductive cycle in bitches. Additional analyses will evaluate the proteins and genes expressed in the organoids when exposed to different hormones to further confirm that the canine endometrial organoids are representative of functional uterine changes that occur in live animals. Afterwards, an additional experiment will be performed that will involve inflammatory stimulation of the organoids to mimic endometritis in the bitch, which causes infertility and can lead on to the development of life-threatening pyometra.