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 2

Report Due: 3/31/2024 **Report Received:** 5/9/2024

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Original Project Description:

Pyometra is a life-threatening disease that affects over 66% of intact older bitches. Bitches are pre-disposed 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 currently in preparation by Dr Alex Horner (AKC funded Reproduction Resident) that will be submitted upon its completion. A second manuscript is expected after completion of Objective 2.

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 under hormonal influence 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 where infection can cause endometritis or life-threatening pus to be produced. This condition is called pyometra. The tissues in these experiments 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. This long culture time is not possible using other cell culture models while maintaining normal function and structure. 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, morphology, and gene changes in response to each hormone treatment. Remarkably, these changes mirror the normal physiological processes that occur during the reproductive cycle in bitches. A manuscript is currently being prepared for publication in the Biology of Reproduction Journal. Afterwards, an additional experiment will be performed that will involve inflammatory stimulation of both healthy and diseased endometrial organoids to mimic endometritis in the bitch, which causes infertility and can lead to the development of life-threatening pyometra. In addition to this experiment, we will also investigate the potential efficacy of a novel therapeutic derived from stem cells in our stimulated canine endometrial organoid model to see if we can reduce or even prevent canine endometrial inflammation. Our goal is to improve the fertility of older bitches that are affected by age-related degenerative changes to their uterus that predispose them to developing endometritis and pyometra.