TITLE

Identification of Early Protein Markers Expressed by Ovarian and Oviductal Surface Epithelial Neoplasias in Laying Hens (Gallus domesticus)

RESEARCH LEADER: Gita Cherian

COOPERATORS: K. Denise Apperson, Christiane Loëhr, Karyn Bird (all with Oregon State University, College of Veterinary Medicine)

SUMMARY

Ovarian adenocarcinoma is a metastatic cancer in which solid tumors develop in the surface epithelium of ovaries and/or oviducts. Early detection remains the most significant issue in devising detection and treatment therapies. The laying hen (*Gallus domesticus*) is a robust animal model for ovarian adenocarcinoma. Tumors in hens have morphology, histopathology, and course of development that is similar to tumors in women. Late-stage tumors in women and laying hens express many of the same proteins.

By the time a laying hen has reached two years of age, she has produced between 300 and 500 eggs and has ovulated about the same number of times as a woman who has reached menopause. This is relevant because there is an established link between incidence of ovarian adenocarcinoma in women and the number of lifetime ovulations. In both hens and women, risk and incidence increase rapidly as ovarian function diminishes.

The use of animal models to evaluate diseases is critical in identifying early disease markers and developing and testing chemotherapies. Early detection is the most significant issue in devising treatment therapies for women with ovarian adenocarcinomas. There are few specific symptoms associated with early stages of the disease and little information on early expression and progression of tumors in women.

OBJECTIVES

Despite the increased use of laying hens as an animal model for ovarian adenocarcinomas, there is no detailed description of the histology of the normal laying hen ovary in either the veterinary medicine literature or the ovarian cancer research literature. Our objective in the first year of this project has been to address that knowledge gap by establishing baselines for the histology of normal laying hen ovaries from which abnormal and cancerous lesions can be more readily identified.

The second phase of the project will take place during the summer of 2017 with tissues and serum collected from a second cohort of 22 hens (24 months of age by June 2017; because the birds are older than those in the first phase, some are expected to have developed detectable adenocarcinomas). Our objective will be further characterization of those ovaries with large numbers of heterophils, a state of chronic inflammation that we propose is a potential precursor to

cancer. With a time-series of serum samples, we hypothesize that laying hen ovaries with abnormal numbers of cortical heterophils will have elevated serum levels of selected cytokines relative to normal ovaries.

PROCEDURES

Ovaries and oviducts were collected from eighteen laying hens (aged 18 months). The tissues were fixed in 10% neutral buffered formalin then processed and embedded in paraffin (Oregon Veterinary Diagnostic Laboratory, Corvallis, OR). Hematoxylin- and eosin-stained, 3-5 micrometer sections were examined by light microscopy for gross and microscopic histopathological characteristics. Immunohistochemical staining was performed on selected samples for pancytokeratin and cytokeratin 7 (Oregon Veterinary Diagnostic Laboratory, Corvallis, OR). The marker pancytokeratin differentiates neoplastic ovarian epithelium from normal epithelium while cytokeratin 7 differentiates Wolffian remnant tissues from normal epithelium.

During the second phase of the project, we will collect and prepare ovary and oviduct tissues from the cohort of 22 hens and examine them for gross and microscopic histological characteristics. Immunohistochemical staining will be performed to differentiate neoplastic ovarian surface epithelium from normal epithelium. We will collect serum samples at three time points (12 months, 18 months, 24 months) and measure concentrations of IL-16 and IL-6.

SIGNIFICANT ACCOMPLISHMENTS TO DATE

In order to fill a knowledge gap, we prepared a detailed description of the histology of the normal laying hen ovary. We also described Wolffian duct remnant tissues that have not been well characterized in avian ovaries before now, although there are published descriptions for these tissues for mammals. These results are being prepared for publication in the peer-reviewed, online journal Veterinary Sciences.

Despite the absence of visually detectable tumors in the birds included in the first phase of the study, ovaries of 10 of the 18 birds had very large numbers of heterophils (the avian equivalent of the neutrophil) in the cortex that were associated with a marked absence of the normal complement of follicles.

We hypothesize that the large numbers of cortical heterophils represent a previously undescribed and unrecognized state of chronic inflammation of the laying hen ovary that may be a precursor to the development of ovarian adenocarcinoma. Using bench-ready, chicken-specific ELISA kits, we will examine serum levels of two cytokines to explore this further. IL-16 is a cytokine that is expressed at elevated levels in the serum of both laying hens and women with ovarian adenocarcinomas, and IL-6 is an inflammatory cytokine that has been explicitly implicated in chemotaxis of avian heterophils.

Apperson, K. D., Loëhr, C., Bird, K., and Cherian, G. Histology of the Normal Laying Hen Ovary. Veterinary Sciences (to be submitted).

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM

K. Denise Apperson received an academic-year grant from the Department of Biomedical Sciences, College of Veterinary Medicine, Oregon State University for $1000 of salary and $640

of laboratory expenses. She also received a $1000 scholarship from the American Association of Avian Pathologists Foundation.

FUTURE FUNDING POSSIBILITIES

K. Denise Apperson will apply again to the American Association of Avian Pathologists (AAAP) for a scholarship. She will also apply to the Department of Biomedical Sciences for more student research funding. She will apply to AAAP and the Poultry Science Association, as appropriate, for travel funds to present the results of this research at one or both annual meetings in 2018.