LUTEINIZING HORMONE RECEPTOR EXPRESSION IN CANINE T-LYMPHOMA CELL LINES

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INTRODUCTION
- Canine lymphoma is a common malignant tumor in dogs.
- Lymphoma is 3-4 times more common in spayed female and neutered male dogs compared to intact females and males. 1,2
- Circulating luteinizing hormone (LH) concentrations are up to 20 times higher in spayed dogs than in intact dogs because of a lack of negative feedback to the anterior pituitary from the gonads (FIGURE 1). 3
- Luteinizing hormone receptors (LHR) are present in reproductive tract and non-reproductive tract tissues (including adrenal cortex, lower urinary tract, skin, thyroid). 4
- LHR have also been found in non-reproductive long-term canine lymphatic tissue. 5
- LHR have also been found in human leukemia cell lines. 6

HYPOTHESES
- We hypothesized that LHR would be present in canine T-cell lymphoma lines.
- We also hypothesized that each cell line would express a different quantity of LHR.

OBJECTIVE
- The objective of this study was to use immunocytochemistry and flow cytometry to measure LHR expression in three cell lines of cultured canine T-lymphoma cells.

MATERIALS & METHODS
- Three T-lymphoma cell lines established from spontaneously occurring canine lymphomas were generously donated from Yamaguchi University.
- Cells were cultured in R10 complete medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37°C in a humidified 5% CO2 incubator.
- Fresh cell culture media was replaced every 2-3 days at a ratio of 1:4 (EMA, CLK) or 1:9 (CLC).
- After reaching 75% confluence, cells were transferred to a 50 mL conical tube and centrifuged at 400 x g for 15 minutes to remove the culture media.
- The supernatant was discarded and the cell pellet was transferred to Eppendorf tubes.
- The cells were washed in ice cold PAB (1% bovine serum albumin and 0.05% sodium azide in phosphate buffered saline) and then resuspended in PAB at final dilution 10,000,000 cells/mL.
- Nonspecific antibody binding was blocked using a Mouse Seroblock FcR (BUF041A, Bio-Rad) at a 1:10 dilution for 10 minutes on ice.
- Cells were washed in PAB, pipetted onto a 96-well plate, and analyzed on a CytoFLEX flow cytometer at the Oregon State University Core Facility.

RESULTS
- All three T-lymphoma cell lines (CLC, EMA, CLK) cell lines expressed LHR.
- The cell population that expressed LHR was smaller in size (forward scatter; FSC) and more granular (side scatter; SSC) (FIGURE 2).
- The CLC cell line had the most cells (45%) that expressed LHR compared to CLK (35%) and EMA (10%).
- There was no positive signal present in any of the negative controls.

DISCUSSION & FUTURE RESEARCH
- This is the first study to show LHR expression in cultured canine lymphoma cells.
- Expression of LHR in normal and neoplastic lymphocytes may explain the higher incidence of lymphoma in dogs that have been spayed and neutered.
- Our laboratory is currently investigating the pro-neoplastic effects of LHR activation (cell proliferation, migration, and invasion) using these cell lines.

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REFERENCES