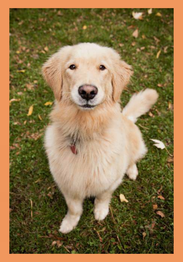

Luteinizing Hormone Receptor Expression in the Canine Coxofemoral and Femorotibial Joints

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INTRODUCTION

- Osteoarthritis (OA) due to canine hip dysplasia (CHD) or anterior cruciate ligament (ACL) injury remains a primary factor contributing to chronic lameness in dogs.[1]
- CHD is a developmental, genetic musculoskeletal disease resulting from the laxity within the coxofemoral joint capsule. Over time, subluxation of the femoral head results in significant changes in the size and shape of both articular joint surfaces.
- ACL injury is an acute, genetic musculoskeletal disease resulting from chronic biomechanical stress within the femorotibial joint. Upon an inciting event, the applied load on the ligament exceeds the magnitude of the load at failure and results in a partial or complete tear of the ACL.[2]
- It is important to note that CHD and ACL injury patients are born with anatomically normal joints, but then develop laxity secondary to intrinsic and extrinsic factors pre- and post-puberty (**Figure 1**).[2-4]
- Surgical sterilization by means of gonad removal (gonadectomy) has become the most common approach towards the management of canine overpopulation and the prevention of reproductive disease.
- However, gonadectomy is associated with several long-term health conditions in dogs, including a 17% increased incidence of CHD and a 2-3 fold increased incidence of ACL injury.[5,6]
- In gonadectomized dogs, plasma luteinizing hormone (LH) concentrations, regulated by gonadal hormone release in intact dogs, are up to 20 times greater.[7]
- LH receptors (LHR) in canine extra-gonadal tissues (e.g. lower urinary tract) and their increased activation post-gonadectomy have been implicated in the pathophysiology of several long-term health problems (e.g. urinary incontinence).

HYPOTHESES & OBJECTIVE

- Based on this information, **it is hypothesized that**:
  1. Luteinizing hormone receptor (LHR) expression will be present in the femoral head subchondral bone (FHSB) and round ligament (RL) of the coxofemoral joint as well as the synovium and ACL of the femorotibial joint.
  2. Gonadectomized dogs will have greater LHR expression than reproductively-intact dogs.
- **Objective:** Characterize LHR expression in the tissues supporting the canine coxofemoral and femorotibial joints utilizing immunohistochemistry for LHR protein detection.

MATERIALS & METHODS

- Tissue samples were collected from 26 dogs postmortem. Information pertaining to the sex, gonadal status, breed, body condition score (BCS), estimated age, and antemortem hindlimb lameness were available from 20 dogs.
  - 12 male dogs (8 neutered, 4 intact)
  - 8 female dogs (6 spayed, 2 intact)
  - Breed (**Figure 2**)
  - BCS ranged from 2-7 (Average: 4.5)
  - Estimated age ranged from 1-13 years
  - Only one dog had evidence of lameness (13 year old neutered male)
- The coxofemoral and femorotibial joint capsules were incised open. The RL, ACL, and synovium were removed and fixed in 10% buffer formalin. The FHSB was removed from the coxofemoral joint using an osteotome and decalcified in Cal-Ex for 7 days before being moved to 10% buffer formalin.
- Similar tissues were combined on the same section with a formalin-fixed skin sample collected from an unrelated canine cadaver (positive control).
- The tissues were paraffin-embedded and sectioned (6 µm) onto charged slides.
- All slides were subjected to routine immunohistochemical techniques using goat polyclonal anti-human primary LHR antibody (SC-26341, Santa Cruz Biotechnology, Dallas, TX) at a 1:50 dilution. Negative controls from each tissue were treated in a similar manner without the primary antibody.
- LHR immunoexpression was detected via bright-field microscopy at 400X magnification:
  - Bone marrow (BM) cells (FHSB) and synoviocytes (synovium) with visible nuclei were counted to determine the percentage of positive-staining cells.
  - Fibrocytes of the RL and ACL were evaluated in a semi-quantitative manner by using the product of scores for positive staining percentage and staining intensity.
  - A student's t-test was used to compare LHR expression between sex and gonadal status groups for each tissue type. Significance was defined as $p<0.05$.

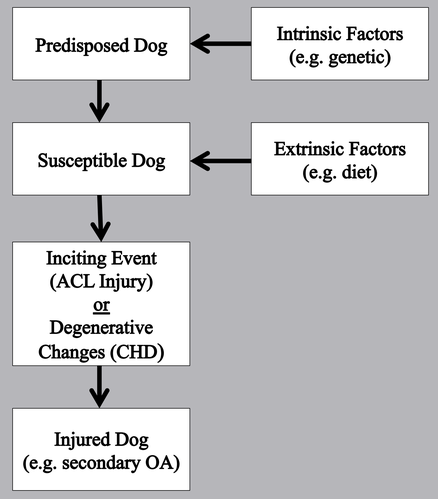

Figure 1. A model of canine injury causation to define CHD and ACL injury with LH/LHR activation as an intrinsic factor. Diagram adapted from description by Meeuwisse and modifications by Bahr and Krosshaug to define human ACL injury mechanisms.[3,4]

Figure 2. Medium and large dog breeds represented by the cadavers used within this study.

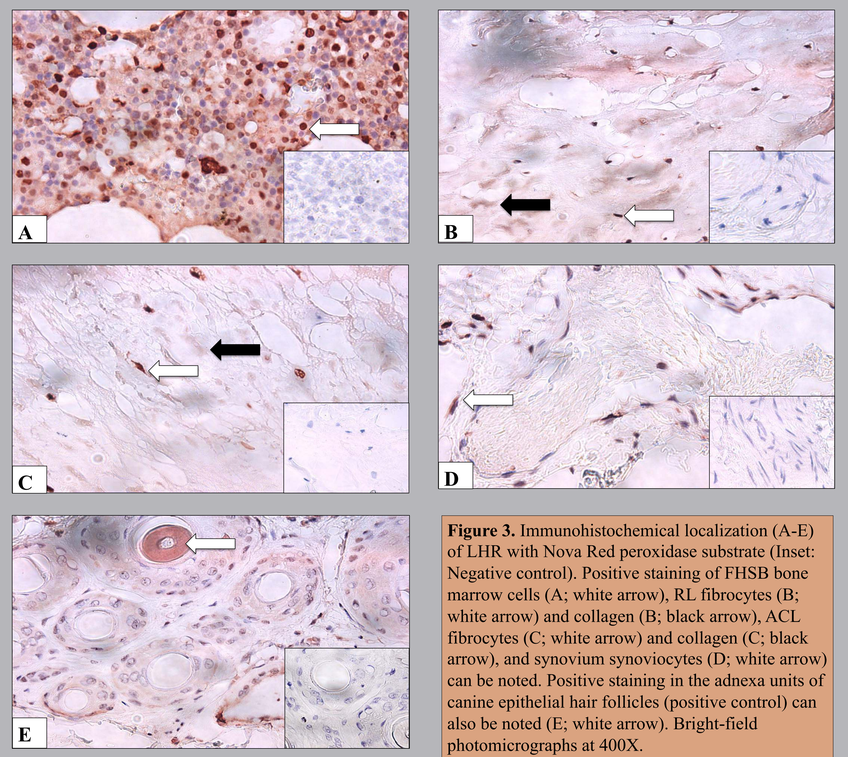

Figure 3. Immunohistochemical localization (A-E) of LHR with Nova Red peroxidase substrate (Inset: Negative control). Positive staining of FHSB bone marrow cells (A; white arrow), RL fibrocytes (B; white arrow) and collagen (B; black arrow), ACL fibrocytes (C; white arrow) and collagen (C; black arrow), and synovium synoviocytes (D; white arrow) can be noted. Positive staining in the adnexa units of canine epithelial hair follicles (positive control) can also be noted (E; white arrow). Bright-field photomicrographs at 400X.

RESULTS

- All FHSB examined expressed LHR. There was no significant difference in LHR expression in BM cells between sex (24.7±22.0% vs. 32.1±34.1%, p=0.32) or gonadal status (27.0±25.4% vs. 27.0±29.1%, p=0.49) (**Figure 3; A**).
- LHR were expressed in nearly 50% of RL tissues examined. Spayed females had significantly greater LHR expression in fibrocytes compared to neutered males (4.33±2.89 vs. 0, p=0.03). All females studied had significantly greater LHR expression in fibrocytes compared to all males studied (4.75±2.5 vs. 0, p=0.004) (**Figure 3; B**).
- LHR were expressed in nearly 75% of ACL tissues examined. The level of expression in fibrocytes did not differ significantly between females and males studied (5.0±1.41 vs. 3.0±4.24, p=0.29) (**Figure 3; C**).
- LHR were expressed in nearly 80% of the femorotibial synovium tissues examined. The level of expression in synoviocytes did not differ significantly between sex (27.7±29.6% vs. 31.3±34.0%, p=0.44) or gonadal status (27.7±29.6% vs. 32.7±37.0%, p=0.43) (**Figure 3; D**).
- None of the negative controls had positive staining for LHR.
- All of the skin samples, serving as the positive experimental control, were positive staining for LHR (**Figure 3; E**).

CONCLUSIONS & FUTURE DIRECTIONS

- As hypothesized, LHR are expressed in the round ligament (RL) fibrocytes and femoral head subchondral bone (FHSB) bone marrow cells of the canine coxofemoral joint as well as the ACL fibrocytes and synovium synoviocytes of the canine femorotibial joint. Except for LHR expression in the RL, there was no significant difference in LHR expression based upon sex or gonadal status.
- Previous studies characterizing the signal transduction pathway of LHR expressed in non-musculoskeletal extra-gonadal tissues have found LH to induce similar secondary effects such as increased blood flow and remodeling processes.[1,8-12]
- Ligand-receptor interactions under supraphysiologic plasma LH concentrations in gonadectomized dogs may augment these effects, create laxity, and explain the increased incidence of CHD and ACL injury in spayed and neutered dogs.[5-7]
- These results provide evidence that LH/LHR activation in the structural support tissues of the canine coxofemoral and femorotibial joints may play a role in the pathophysiology of acquired musculoskeletal diseases post-gonadectomy.
  - Gonad-sparing sterilization procedures (e.g. hysterectomy, vasectomy) in predisposed breeds may be an alternative sterilization method for consideration.
- In vitro research of these isolated ligaments in a tissue bath is needed to determine the functionality of these receptors. Additionally, clinical research is needed to compare the musculoskeletal benefits of lowering LH in spayed and neutered dogs using gonadotropin-releasing hormone (GnRH) agonists or vaccines.

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