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Luteinizing hormone receptor expression in the canine femoral head ligament, hyaline cartilage, and subchondral bone

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In the United States, surgical sterilization has become a common tool for managing canine overpopulation as well as preventing reproductive diseases in dogs (e.g. mammary cancer, prostate hyperplasia/prostatitis). However, surgical sterilization with gonadectomy is associated with long-term health problems in dogs. Gonadectomy significantly increases the incidence of canine hip dysplasia by 1.5-2 fold.1 In the intact dog, luteinizing hormone (LH) secreted from the anterior pituitary gland stimulates the synthesis of gonadal hormones (e.g. testosterone, estrogen), which negatively feedback to regulate LH secretion. Conversely, in the gonadectomized dog, there is no negative feedback due to the absence of gonads to complete the feedback loop. Thus, LH concentrations can increase to over 30 times higher than in intact adult dogs. Although the main role of LH remains within the gonads, LH receptors (LHR) are present in many non-reproductive tissues (e.g. bladder, skin, thyroid) and have been associated with several problematic conditions (e.g. urinary incontinence, puppy coat syndrome, hypothyroidism). Based upon the increased incidence of hip dysplasia in gonadectomized dogs, we hypothesized that there are LHR in the canine coxofemoral joint, specifically the femoral head ligament, hyaline cartilage, and subchondral bone. The objective of the study was to use immunohistochemistry to determine if LHR were present in these tissues. The femoral head was removed from six dogs postmortem using an osteotome. The femoral head ligament was dissected free using a scalpel blade and hyaline cartilage adjacent to the ligament insertion site was shaved off using the same scalpel blade. These tissues were fixed in 10% buffer formalin. The femoral head was decalcified in Cal-Ex (Fisher Scientific, Waltham, MA) for 7 days and then moved to formalin. Similar tissues from all six dogs were combined on the same section with a formalin-fixed canine testicle collected from an unrelated dog during a routine castration (positive control). These tissues were then paraffin-embedded and sectioned (6 µm) onto charged slides. All slides were deparaffinized, rehydrated, and subjected to heat-induced epitope retrieval (#S1700, Dako, Carpinteria, CA). Endogenous peroxidase activity was inactivated with 3% hydrogen peroxide and nonspecific binding was blocked with 1% horse serum. Goat polyclonal anti-human LHR antibody (SC-26341, Santa Cruz Biotechnology, Dallas, TX) was applied at a 1:50 dilution. Negative controls from each tissue were treated in the same way except in the absence of primary antibody. Slides were then reacted with biotinylated horse anti-goat IgG (Vector Laboratories, Burlingame, CA) and incubated with preformed avidin-biotin-peroxidase complex (#PK6105, ABC kit, Vector Laboratories) followed by Nova Red Peroxidase substrate (#SK4800, Vector Laboratories). Slides were counter-stained with hematoxylin, dehydrated, and mounted. Immunoexpression of LHR was detected utilizing bright-field microscopy at 400X magnification. Although the number of positive cells varied between dogs, LHR expression was present in all three tissue types examined. The etiopathogenesis for the increased incidence of hip dysplasia in gonadectomized dogs remains unknown. However, the results from this preliminary study suggest that persistent LHR activation in such structural support tissues within the hip joint may play a role. LHR activation is known to stimulate nitric oxide release,2 which in the coxofemoral joint of predisposed breeds could result in excessive joint laxity and thus the gradual degeneration and symptoms associated with hip dysplasia.