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Understanding the effects of sustained supraphysiologic concentrations of luteinizing hormone in gonadectomized dogs: What we know and what we still need to learn

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ABSTRACT

Removal of the gonads with surgical sterilization results in a loss of negative feedback to the hypothalamus and anterior pituitary. The sustained supraphysiologic luteinizing hormone (LH) concentrations in gonadectomized dogs can significantly alter organ function and even induce neoplastic changes. For example, gonad removal has a profound effect on thyroid function and is reported to be the most significant cause for the development of hypothyroidism in dogs. Thirty percent more gonadectomized dogs develop hypothyroidism compared with intact dogs. Within the canine thyroid, LH receptors are co-localized with thyroid stimulating hormone (TSH) receptors. Continuous LH receptor activation in gonadectomized dogs may interfere TSH receptor function by consuming second messengers involved in G-protein receptor cell signaling, preventing the action of TSH when it binds to its receptor in the thyroid, resulting in hypothyroidism. The incidence of anterior cruciate ligament ruptures is significantly increased following gonad removal independent of breed, sex, weight or body condition. Luteinizing hormone receptors are expressed in the cruciate ligament and continuous LH receptor activation may increase laxity in these ligaments, resulting in joint instability. Both male and female gonadectomized dogs are at a significantly increased risk for lymphoma and hemangiosarcoma. Luteinizing hormone receptors are also abundant in these tissues. Research in four canine hemangiosarcoma cell lines found that LH receptor activation induces cell proliferation. In addition, research in three canine T-cell lymphoma cell lines found that LH receptor activation induces cell proliferation, adhesion, and invasion as well as increases LH receptor expression. Research is needed to determine if LH reducing strategies using gonadotropin releasing hormone agonists will increase remission times in gonadectomized dogs with LH receptor-positive tumors. In conclusion, among the non-reproductive functions of gonads, suppression of LH secretion and resulting LH receptor overexpression appear necessary in maintaining endocrine, musculoskeletal, and anti-neoplastic health.

1. Introduction

Reproductive sterilization via gonadectomy has been used for millennia in humans as a form of punishment and to facilitate subservience [1,2]. By the time of Aristotle in the fourth century BC, the physiological consequences of gonadectomy in men and boys were well-documented [3]. Because gonadectomy was performed pre-pubertally, these men grew taller than their unsterilized counterparts, due to a delay in the long bone growth plate closure that is normally initiated by the rise in gonadal steroid hormones at puberty [4]. It is important to mention that in addition to the physical differences observed, pre-pubertal gonadectomy in humans also results in grossly enlarged pituitary glands [5].

Similar to humans, pre-pubertally gonadectomized male and female dogs have significantly delayed physeal closure and grow taller than their unsterilized counterparts [6]. The effects of these skeletal changes can predispose pre-pubertally gonadectomized dogs to musculoskeletal problems later in life (e.g., slipped capital femoral epiphysiolysis) [7]. In addition to these skeletal changes, gonadectomy results in a multitude of physical, metabolic, endocrinologic, behavioral and anti-neoplastic changes in dogs that can cause in long-term health problems. The pathophysiology for these health problems can be traced back to activation of luteinizing

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hormone (LH) receptors in these non-reproductive tissues, resulting from sustained exposure to supraphysiologically elevated LH concentrations from lack of feedback.

In the mature intact mammal, the hypothalamus secretes gonadotropin-releasing hormone (GnRH), which stimulates the anterior pituitary gland to release LH. Luteinizing hormone stimulates the secretion of gonadal steroid hormones (testosterone in males and estradiol/progesterone in females). These gonadal steroid hormones then negatively feedback directly to the anterior pituitary as well as indirectly through to the hypothalamus to maintain circulating LH concentrations below 1 ng/mL. However, in the mature gonadectomized mammal, the absence of negative feedback results in circulating LH concentrations up 100 ng/mL [8].

Receptors for LH are widely disseminated throughout the body, including but not limited to the skin, bladder and urethra, thyroid gland, adrenal cortex, ligaments and bone, vascular endothelial cells and smooth muscle, and lymphocytes [9]. There are several ways LH receptor activation regulates cell function. For example, LH receptor activation leads to both protein kinase A (PKA) activation [10] and PKA-dependent ERK1/2 activation [11], which can increase protein synthesis and nitric oxide release. In addition, activation of LH receptors can induce cell proliferation through an ERK-dependent pathway [12]. However, less is known about how LH receptor activation in non-reproductive tissues elicits various disease states and more research in these areas is needed.

2. Urinary incontinence

2.1. What we know

Urinary incontinence is a common long-term health complication of gonad removal in female dogs, with a reported incidence of up to 30% [13–16]. The association between urinary incontinence and gonad removal in female dogs was first described by Joshua (1965) [17]. Reducing circulating LH concentrations can restore urinary continence in gonadectomized incontinent females using estrogens [15,18–21], GnRH agonists [22,23], or GnRH immunization [24,25]. Reducing LH concentrations is relevant because LH receptors are abundantly expressed in the epithelial cells and smooth muscles of the canine lower urinary tract (bladder neck and urethra) [26,27]. In addition, gonadectomy significantly increases LH receptor gene expression in the lower urinary tract in both sexes [28]. This is important to note because the theory of receptor homeostasis states that as hormone (ligand) concentration increases, receptor density (expression) decreases.

2.2. What we still need to learn

The role of LH receptor activation in the etiopathogenesis of urinary incontinence in gonadectomized females remains unclear. For example, how does the dog's age at the time of gonadectomy influence the risk for incontinence. Results are mixed showing an increased risk for incontinence when gonadectomized before 12 weeks old [16] and when gonadectomized after puberty [29]. Also, how does the dog's body weight and/or breed influence the risk for incontinence. Female dogs weighing over 15 kg are about seven times more likely to become incontinent following gonadectomy [30]. Boxers, Dobermans, Giant Schnauzers, Irish Setters, Rottweilers, Springer Spaniels, and Weimaraners are over-represented, while other large breed dogs (e.g., German Shepherds, Labrador Retrievers) are rarely affected [13,15,29,31–33]. In addition, why is it that some gonadectomized bitches develop incontinence immediately, while others do not develop incontinence until years later [13,33,34]. And last, does LH receptor activation in the lower urinary tract reduce bladder contractility by increasing the collagen to smooth muscle content? If so, is this change reversible?

3. Hypothyroidism

3.1. What we know

Hypothyroidism is a common endocrine disorder in which the thyroid gland does not produce sufficient quantities of thyroid hormone [35,36]. Gonad removal has a profound effect on thyroid function [37] and is the most significant cause for the development of hypothyroidism in dogs [38]. Thirty percent more gonadectomized dogs develop hypothyroidism compared with unaltered dogs [39]. In addition, the concentrations of thyroxine in gonadectomized dogs were significantly lower in both sexes when compared with unaltered dogs [40]. It is interesting to note that women who have undergone gonadectomy are also at an increased risk for developing hypothyroidism [41].

Previous studies in humans have shown that LH receptors are expressed in normal and adenomatous thyroid glands [42]. Our laboratory demonstrated the expression of LH receptors in normal canine thyroid glands [43]. Of particular importance, our research revealed that LH receptors were co-localized with thyroid stimulating hormone (TSH) receptors on canine thyrocytes.

3.2. What we still need to learn

The role (if any) of LH receptor activation in the etiopathogenesis of hypothyroidism in gonadectomized dogs has not been studied yet. Activation of thyroid LH receptors may interrupt with intracellular signaling from TSH receptor activation because both of these G-protein coupled receptors utilize adenylate cyclase and cAMP [44]. The net result of TSH receptor interference would be decreased thyroxine secretion from thyrocytes [45]. Utilizing an *in vitro* model, the thyroxine secretion response to TSH receptor activation could be measured in cultured canine thyroid tissue slices or in three-dimensional multicellular thyrocyte spheroids with or without concurrent LH receptor activation [46–48]. In addition, clinical trials could be conducted measuring thyroxine concentrations in gonadectomized dogs before and after treatment with a GnRH agonist to downregulate LH secretion; particularly if reducing LH concentrations would restore normal thyroid function in gonadectomized dogs with hypothyroidism.

4. Anterior cruciate ligament rupture

4.1. What we know

The anterior cruciate ligament serves to prevent cranial displacement of the tibia relative to the femur, to limit internal rotation of the tibia relative to the femur, and to prevent stifle hyperextension [49,50]. Anterior cruciate ligament rupture is musculoskeletal disorder that initially involves the degeneration of the cranial cruciate ligament, which leads to a partial rupture and then progresses to a complete rupture following an unspectacular traumatic event [51,52]. Most dogs that develop anterior cruciate ligament ruptures are born with normal stifle joints but then develop the tendency for anterior cruciate ligament rupture secondary to intrinsic and/or extrinsic factors. Gonad removal significantly increases the prevalence of anterior cruciate ligament rupture [53], doubling the occurrence reported for unaltered dogs [54], with an incidence as high as 5.1% and 7.7% in males and females, respectively [55]. Prepubertal gonad removal delays tibial growth plate closure, which extends the length of tibia and the steepness of the tibial plateau [56,57]. Increased steepness of the tibial plateau can increase the cranial tibial thrust, which is a risk

for anterior cruciate ligament rupture [58,59].

Despite the skeletal deformations that occur with prepubertal gonad removal, gonad removal postpubertally still results in an increased risk for anterior cruciate ligament rupture [55]. There is evidence from humans that reproductive hormones play a role in altering anterior cruciate ligament laxity [60,61]. This was supported by research that demonstrated the expression of LH receptors within intact [62] and ruptured canine anterior cruciate ligaments (Dr. Garry Bright, veterinary acupuncturist, Cooroy, Queensland, Australia), unpublished work).

4.2. What we still need to learn

Increased LH receptor activation in the anterior cruciate ligament following gonad removal may increase laxity and result in joint instability, predisposing to the higher occurrence of ligament ruptures in gonadectomized dogs. Within the reproductive tract, activation of the LH receptors increases local nitric oxide release, which facilitates vasodilation and angiogenesis [63]. In addition to inducing laxity, increased nitric oxide within ligaments may alter collagen expression [64,65]. Altered collagen expression within the cruciate would decrease stress tolerance with increased load to the joint [66]. Decreases in fibroblast cell populations, increase in chondroid metaplastic cell populations, and extracellular matrix disruption have been described in morphological evaluation of ruptured cruciate ligaments [67]. *In vitro* functional studies should be designed to measure cruciate laxity with and without the addition of human chorionic gonadotropin or recombinant canine LH. For example, a differential variable reluctance transducer strain gauge could be used on specimens of normal and abnormal canine cruciate to determine if cruciate LH receptor are functional [68]. Last, dogs with a unilateral cruciate tear are significantly more likely to tear the cruciate ligament in the contralateral limb [69,70]. A prospective case-controlled clinical study could be designed in gonadectomized dogs where LH concentrations are reduced with a GnRH agonist implant administered at the time of the unilateral ligament repair. The survival time of the contralateral cruciate ligament could be compared between a group not receiving a GnRH agonist implant.

5. Hemangiosarcoma

5.1. What we know

Hemangiosarcoma is a rapidly growing, highly invasive cancer arising from the lining of blood vessels and occurring almost exclusively in dogs [71]. Primary tumors can arise in any vascular tissue, but the spleen and heart are the most common locations for hemangiosarcoma to develop [72–74]. Because of its aggressive biological behavior, canine hemangiosarcoma is associated with a poor prognosis [71]. Despite surgical removal of the primary tumor, median survival time in dogs with splenic hemangiosarcoma is two months or less [73,74]. Adding chemotherapy with vincristine, doxorubicin, epirubicin, cyclophosphamide, anthracycline or metronomic-based protocols may prolong survival medians to 6–12 months [74–76].

Dogs affected by hemangiosarcoma are typically older than 5 years but the disease can occur at younger ages [73,77]. German Shepherds, Golden Retrievers, and Labrador Retrievers are overrepresented compared to other breeds, making up 34.33% of all purebred dogs with splenic hemangiosarcoma [73,74,77]. In addition to age and breed, gonad removal also increases the likelihood for developing splenic hemangiosarcoma. In comparison to unaltered female dogs, gonadectomized female dogs have 2 to 10 times the risk for developing hemangiosarcoma [55,78,79].

Earlier studies have confirmed the presence of LH receptors in vascular endothelial and smooth muscle cells [80,81]. Our laboratory has demonstrated the expression of LH receptors in neoplastic endothelial cells of splenic, cardiac, cutaneous and dermal canine hemangiosarcomas *in situ* as well as in isolated canine hemangiosarcoma cells [82,83]. Recently, we demonstrated through a cell proliferation assay that hemangiosarcoma LH receptors are functional in response to activation with recombinant canine LH [84].

5.2. What we still need to learn

In both dog and man, nearly all cardiac hemangiosarcomas originate from the right atrium (more specifically, the right auricle) [85–89]. In cattle, foci of undifferentiated connective tissue exist in the right atrial subepicardium [90], which could be an explanation for the development of hemangiosarcoma in this area. However, based upon the cell proliferation responses to LH activation in isolated splenic hemangiosarcoma cells, it is warranted to isolate cells specifically from the right auricle to compare the LH receptor expression between other areas of the heart.

Improving survival times is a goal of any cancer therapy. This is particularly difficult given the aggressive nature of this cancer. However, in cases where removal of the primary tumor is possible (spleen, skin) and there are no visible metastases, reducing circulating LH concentrations in gonadectomized dogs may reduce residual tumor cell proliferation *in vivo*. A prospective case-controlled clinical study could be designed in gonadectomized dogs where LH concentrations are reduced with a GnRH agonist implant administered at the time of the tumor removal. The remission time could be compared to controls who still have high circulating LH concentrations.

6. Lymphoma

6.1. What we know

Lymphoma is a cancer of lymphocytes and/or lymphoid tissues. Lymphoma is the most common cancer diagnosed in dogs, accounting for up to 24% of all canine cancers [91]. Predisposed breeds include Boxers, Bull Mastiffs, Basset Hounds, St. Bernards, Bulldogs, German Shepherds, Golden Retrievers, Labrador Retrievers, Rottweilers, Cocker Spaniels, Beagles, and Shih Tzus [92]. In addition to breed, gonad removal increases the risk for lymphoma. Lymphoma is three to four times more common following gonadectomy [55,79].

LH receptors are present in lymphocytes and lymphoid tissue (medulla of thymus) [93,94]. Our laboratory demonstrated that the percentage of circulating LH receptor-positive T-lymphocytes was significantly higher in gonadectomized dogs compared to unaltered dogs [94]. In addition, we demonstrated that 12.4% of cells within canine neoplastic lymph nodes express LH receptors and up to 45% of isolated neoplastic T-lymphocytes express LH receptors [94]. To determine if LH receptors in neoplastic T-lymphocytes were functional, a series of experiments were performed. In response to LH receptor activation with human chorionic gonadotropin and/or recombinant canine LH, LH receptor expression increases [95] and neoplastic T-cell lymphocytes proliferate [96], adhere [97] and migrate through an endothelial monolayer [98].

6.2. What we still need to learn

While the function of the LH receptor in canine neoplastic T-cell lymphocytes has been well-described *in vitro*, clinical studies are needed to apply these findings *in vivo*. Similar to hemangiosarcoma, prospective case-controlled trials to determine if reducing

circulating LH concentrations with a GnRH agonist implant will increase remission times are needed.

7. Conclusion

Although commonly performed on dogs in the United States and other parts of the world, reproductive sterilization with gonad removal should not be considered a *routine* procedure. Any surgery that removes the gonads changes the animal in many ways. Gonads should no longer be considered mere gamete-producing or ancillary sex organs but rather necessary endocrine glands for normal metabolic, endocrinologic, musculoskeletal, and anti-neoplastic health. Furthermore, the injurious effects of gonad removal on role of LH receptor activation in dogs with sustained and elevated circulating LH concentrations warrants continued funding for future research.

Declaration of competing interest

The author has no competing interests to declare.

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