



Innovation to save lives.

ASSESSING THE RISK/BENEFIT PROFILE OF SURGICAL STERILIZATION: LABORATORY & EPIDEMIOLOGICAL APPROACHES

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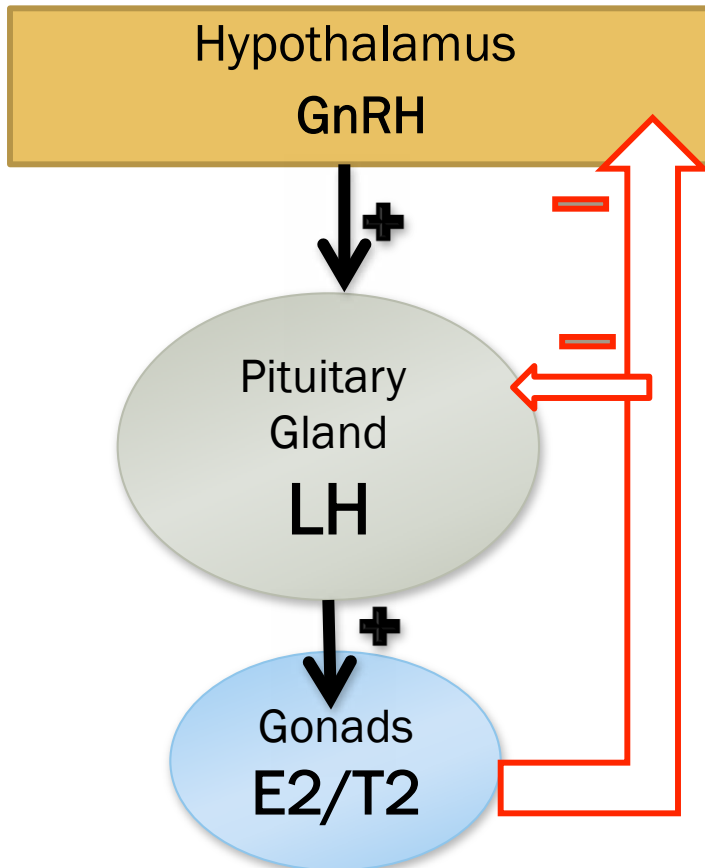
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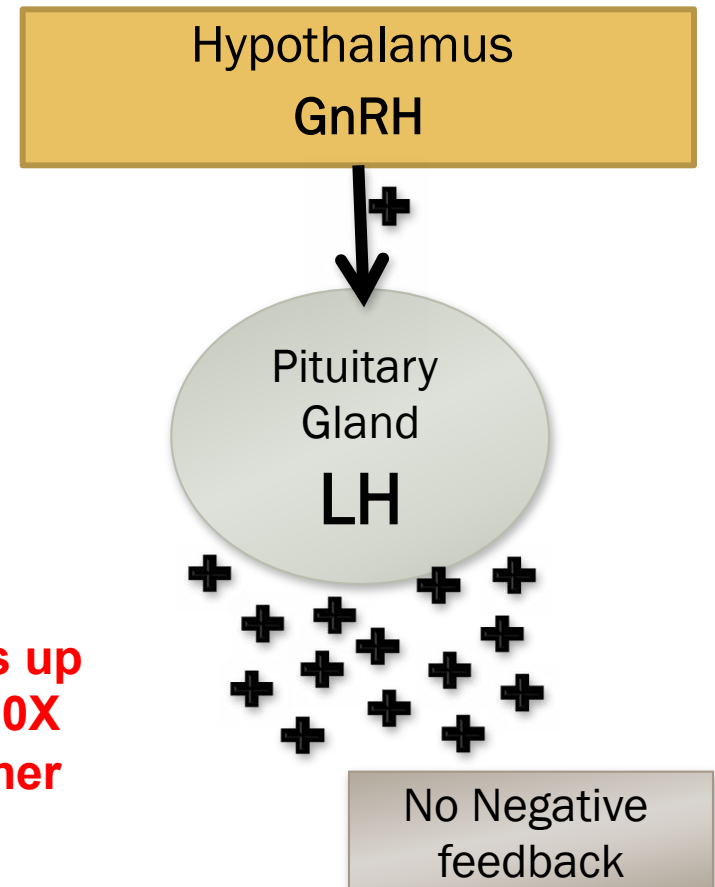


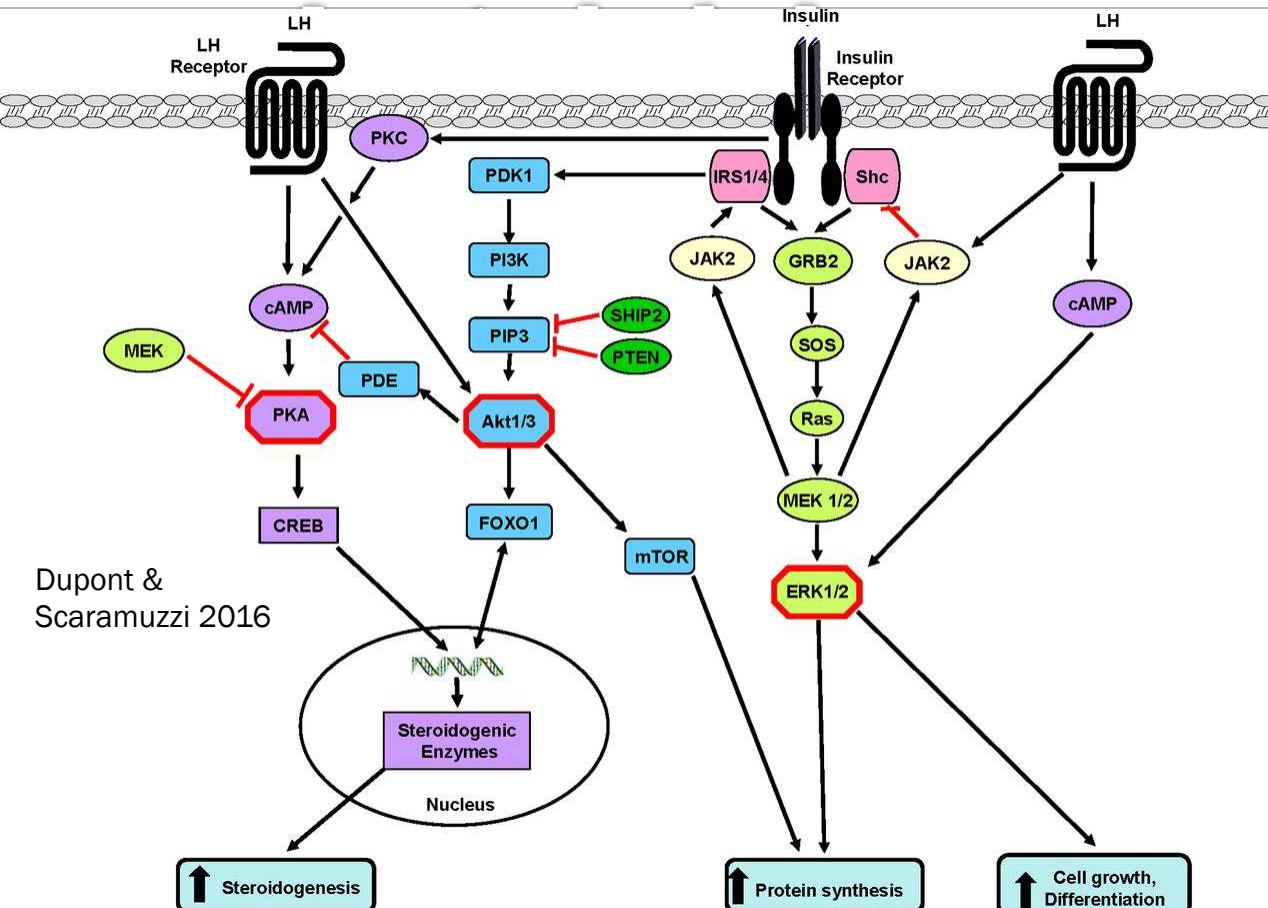
Hypothalamic-Pituitary-Gonadal Axis

∞ With gonads

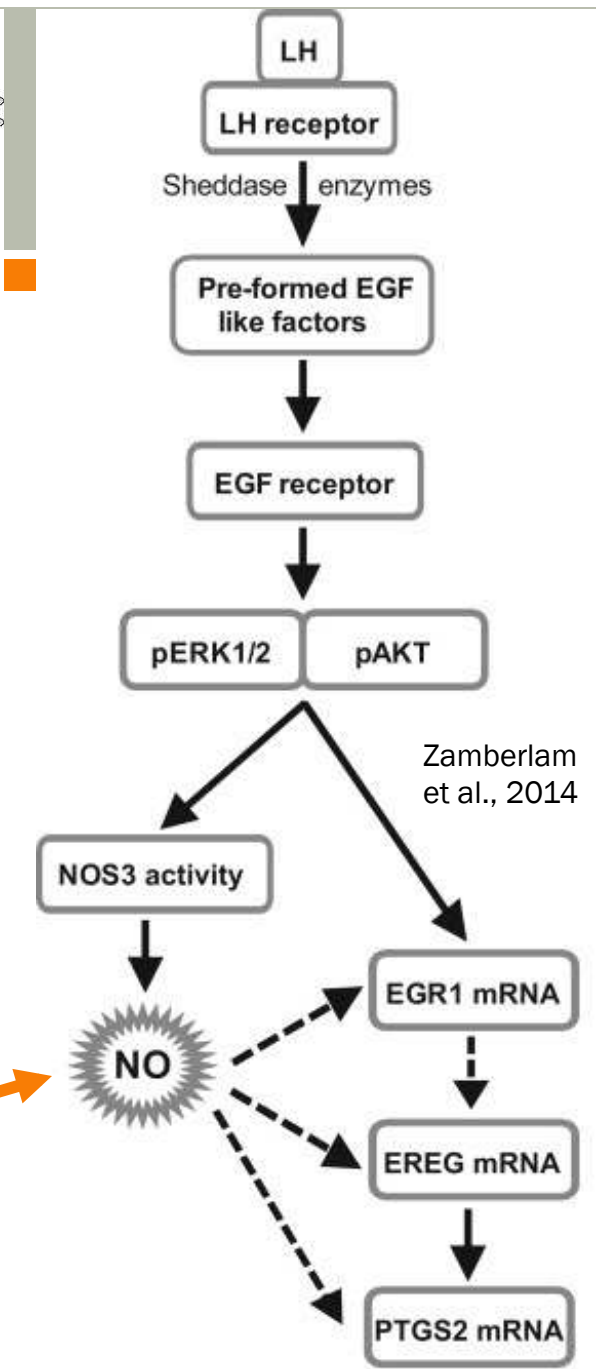


∞ Without gonads





Dupont & Scaramuzzi 2016



Zamberlam et al., 2014

When LH binds to its receptor, it induces cell division & stimulates nitric oxide release

• Greene & Ginther, 2011

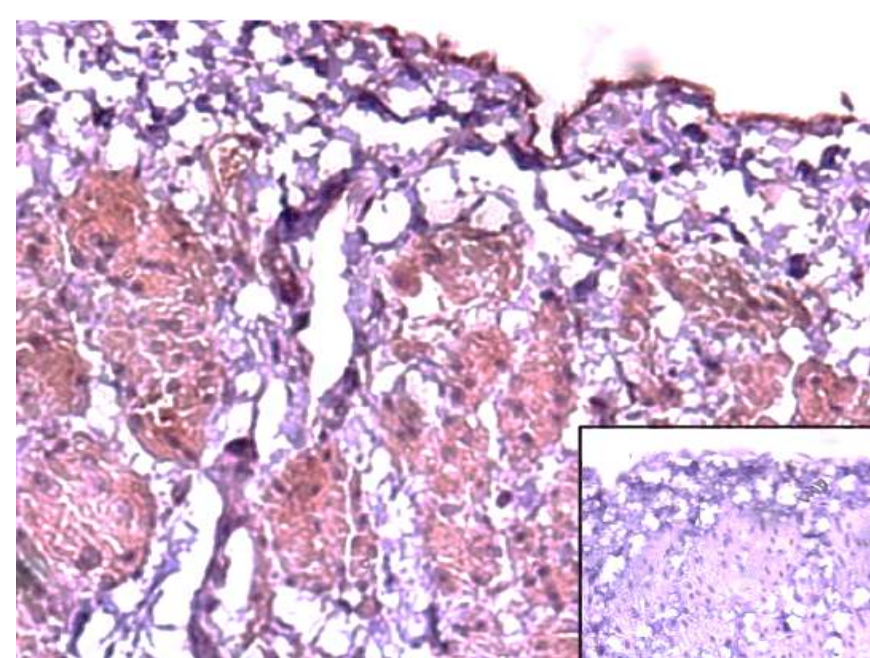
LHR in Non-Reproductive Tissues

Non-Reproductive Tissues	Species	References
Adrenal cortex	Dog, human, rat, rhesus macaque	Papadopoulos et al., 1991; Galac et al., 2010; Nicolini et al., 2014; Lasley et al., 2015
Blood vessels (endothelial cells, vascular smooth muscle cells)	Human	Reshef et al., 1990; Lei et al., 1993; Bukovsky et al., 2003
Brain (hippocampus, hypothalamus, cerebellum, brain stem, cortex)	Guinea pig, rat	Lei et al., 1993; Wahjoepramono et al., 2011
Fibroblasts	Human	Bukovsky et al., 2003
Gastrointestinal tract (enteric neurons, smooth muscle)	Human, rat	Hammar et al., 2012; Sand et al., 2013, Ducker et al., 1996
Lower urinary tract (bladder and urethra)	Dog, human	Welle et al., 1999; Ponglowhapan et al., 2007; Ponglowhapan et al., 2008; Schwalenberg et al., 2012
Lymphoid tissues (thymus and lymphocytes)	Hamsters, human	Maria, 1998; Seiki et al., 1990
Skin (epidermis, hair follicle, sebaceous glands, sweat glands)	Dog, human	Welle et al., 2006; Venencie et al., 1999
Striated muscle cells	Human	Bukovsky et al., 2003
Thyroid gland	Human	Liu et al., 2014

Urinary Bladder

- ∞ LH receptors are expressed in all regions the canine lower urinary tract (body & neck of the bladder, proximal & distal urethra) & in all tissue layers (epithelium, sub-epithelial stroma & muscle)

- Welle MM, Reichler IM, Barth A, Forster U, Sattler U, Arnold S. Immunohistochemical localization & quantitative assessment of GnRH-, FSH-, & LH-receptor mRNA Expression in canine skin: a powerful tool to study the pathogenesis of side effects after spaying. *Histochem Cell Biol.* 2006 Nov;126(5):527-35
- Ponglowhapan S, Church DB, Scaramuzzi RJ, Khalid M. Luteinizing hormone & follicle-stimulating hormone receptors & their transcribed genes (mRNA) are present in the lower urinary tract of intact male & female dogs. *Theriogenology.* 2007 Jan 15;67(2):353-66



Urinary Incontinence

Overall incidence in spayed females: 5-50%

- Arnold, 1997; Stocklin-Gautschi et al., 2001; Angioletti et al., 2004; Spain et al., 2004

Higher in medium & large breed dogs (>30 lb)

Age at time of spaying does not affect likelihood of developing incontinence

Evaluation of the prevalence of urinary incontinence in spayed female dogs: 566 cases (2003–2008)

Kara M. Forsee, DVM; Garrett J. Davis, DVM, DACV; Emily E. Mouat, DVM; Katharine R. Salmeri, DVM, DACV; Richard P. Bastian, PhD

Objective—To determine the prevalence of urinary incontinence in spayed female dogs and categorize affected dogs by age at time of ovariectomy, number of litters prior to ovariectomy, body weight, treatment of affected dogs, and severity of incontinence and to determine associations among these variables.

Design—Retrospective case series.

Animals—566 ovariectomized dogs.

Procedures—An attempt was made to contact owners of 912 dogs ovariectomized between January 2003 and January 2008 to discuss presence or absence of urinary incontinence. The actual number of responders was 566. Those owners with incontinent pets received a questionnaire further assessing degree of incontinence, diagnostic testing, treatment, and history.

Results—The prevalence of acquired urinary incontinence was determined to be 5.12% (29/566 dogs) on the basis of results of phone surveys and questionnaires. There was no significant difference in the age at time of ovariectomy between incontinent and continent groups. A significant association was found between body weight and incontinence, with incontinence rates higher among larger (> 15 kg [33.1 lb]) dogs. Larger dogs were approximately 7 times as likely (OR, 7.2 [95% confidence interval, 2.5 to 21.1]) to develop acquired urinary incontinence, compared with small dogs (< 15 kg).

Conclusions and Clinical Relevance—Although acquired urinary incontinence in female dogs is known to be associated with ovariectomy, the prevalence in this study was low. (J Am Vet Med Assoc 2013;242:959–962)

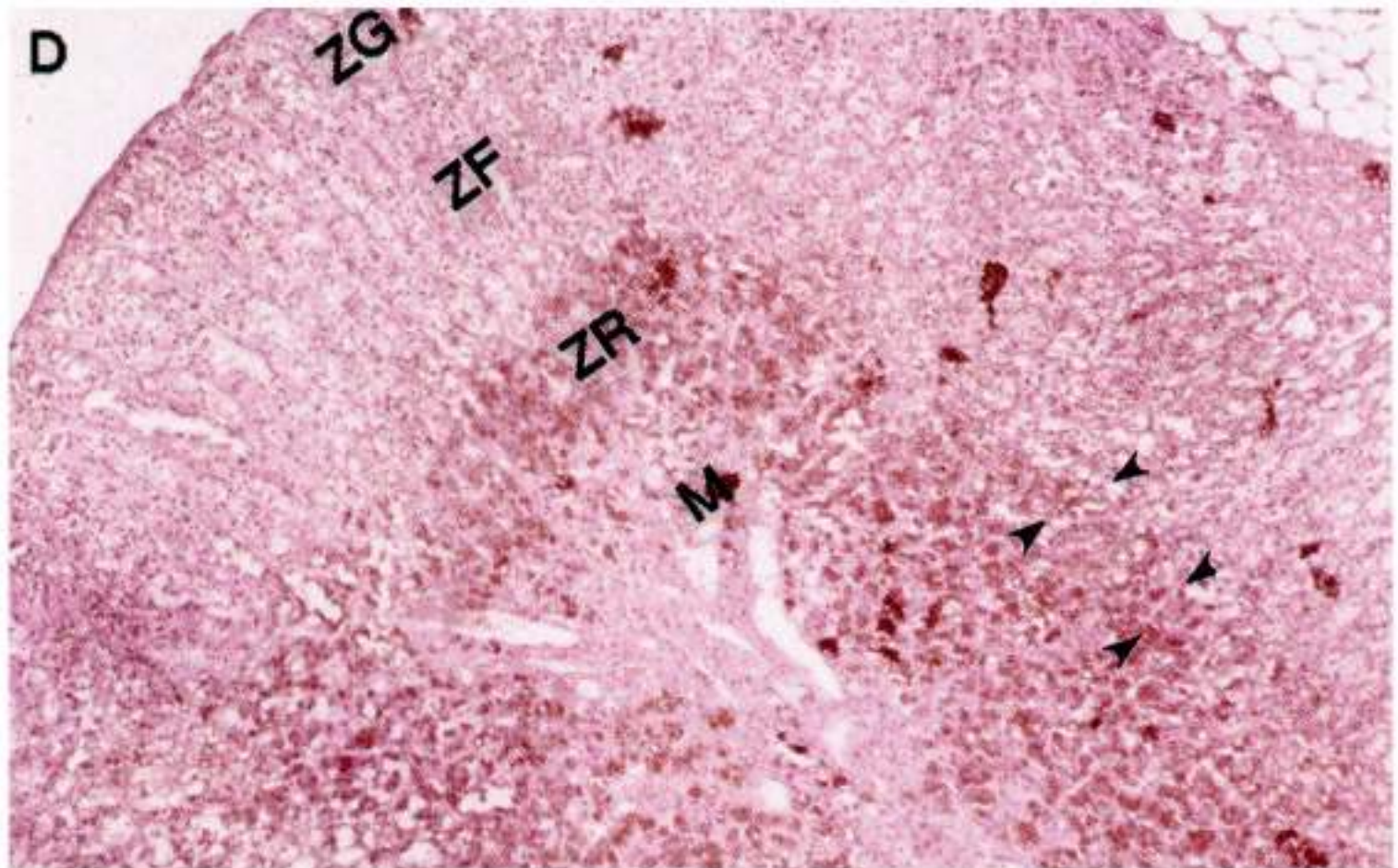
Urinary Incontinence

- ✎ Gonadectomized female dogs with urinary incontinence have a significantly higher number of LH receptors in the lower urinary tract compared to unaltered females
 - Coitet et al., 2009
- ✎ Urinary continence can be restored in gonadectomized females by reducing circulating LH concentrations using:
 - Estrogen
 - Rosin et al., 1981; Hill et al., 2012; Veronesi et al., 2009; Angioletti et al., 2004; Mandigers & Nell, 2001
 - GnRH agonist (deslorelin)
 - Reichler et al., 2003; Reichler et al., 2006
 - GnRH immunization
 - Donovan et al., 2013; Donovan et al., 2014

Adrenal Cortex

∞ Human

Dogs (Papadopoulos 1991), primates & rodents
(Galac 2010; Nicolini 2014; Lasley 2015)



Pabon et al.,
1996)

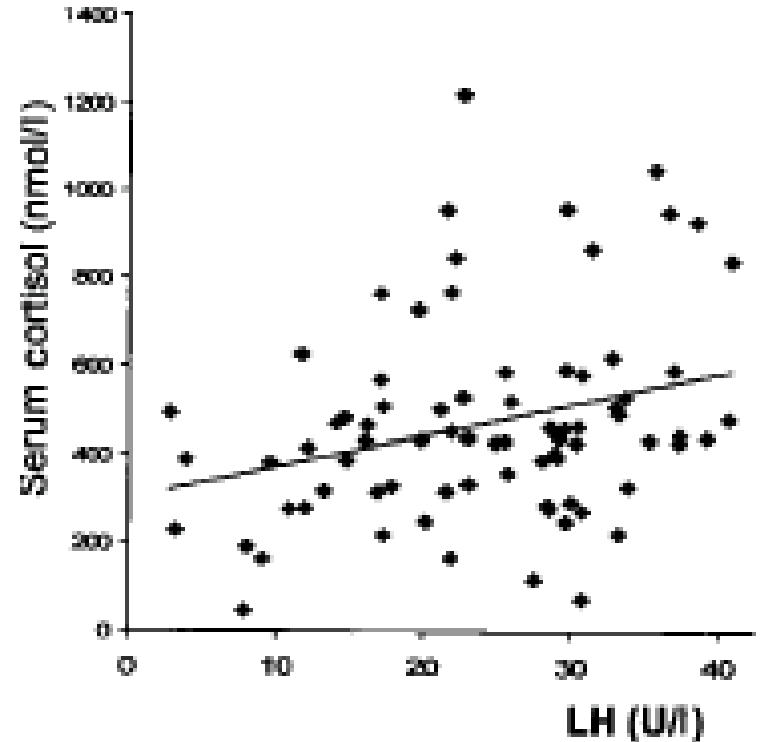
Hyperadrenocorticism (Cushing's)

∞ In postmenopausal women, there is a positive relationship between LH & cortisol concentrations

- Alevizaki et al., 2006

∞ Furthermore, LH receptors on the adrenal gland have been shown to mediate ACTH-independent Cushing's syndrome

- Saxena & Seely, 2012



Hyperadrenocorticism (Cushing's)

☞ In dogs, gonadectomy is associated with a significantly increased risk for hyperadrenocorticism

☞ In ferrets, neutering is also an important risk factor for hyperadrenocorticism

Disease	Neutered Female	Neutered Male
Ocular		
Early onset Cataracts	0.42 + 0.04	0.66 + 0.07
Lens luxation	1.13 ± 0.17	1.19 ± 0.20
Orthopedic		
Elbow dysplasia	0.91 ± 0.09	0.89 ± 0.07
Hip Dysplasia	0.93 ± 0.07	0.96 ± 0.06
Intervertebral disk disease	1.70 + 0.10	1.06 ± 0.04
Patellar luxation	0.99 ± 0.06	0.95 ± 0.06
Ruptured Anterior Cruciate Ligament	3.18 + 0.45	2.32 + 0.28
Cancer		
Hemangiosarcoma	3.18 + 0.73	1.39 + 0.17
Hyperadrenocorticism (Cushings)	4.56 + 0.76	2.02 + 0.24
Lymphoma	2.25 + 0.28	1.20 + 0.09
Mast cell tumor	2.78 + 0.33	1.25 + 0.11
Osteosarcoma	2.53 + 0.47	1.62 + 0.20



Virbac
ANIMAL HEALTH

NOT APPROVED BY FDA - Legally marketed as an FDA Indexed Product under MIF 900-013.
FOR USE IN FERRETS ONLY. Extra-label use is prohibited.
This product is not to be used in animals intended for use as food for humans or other animals.

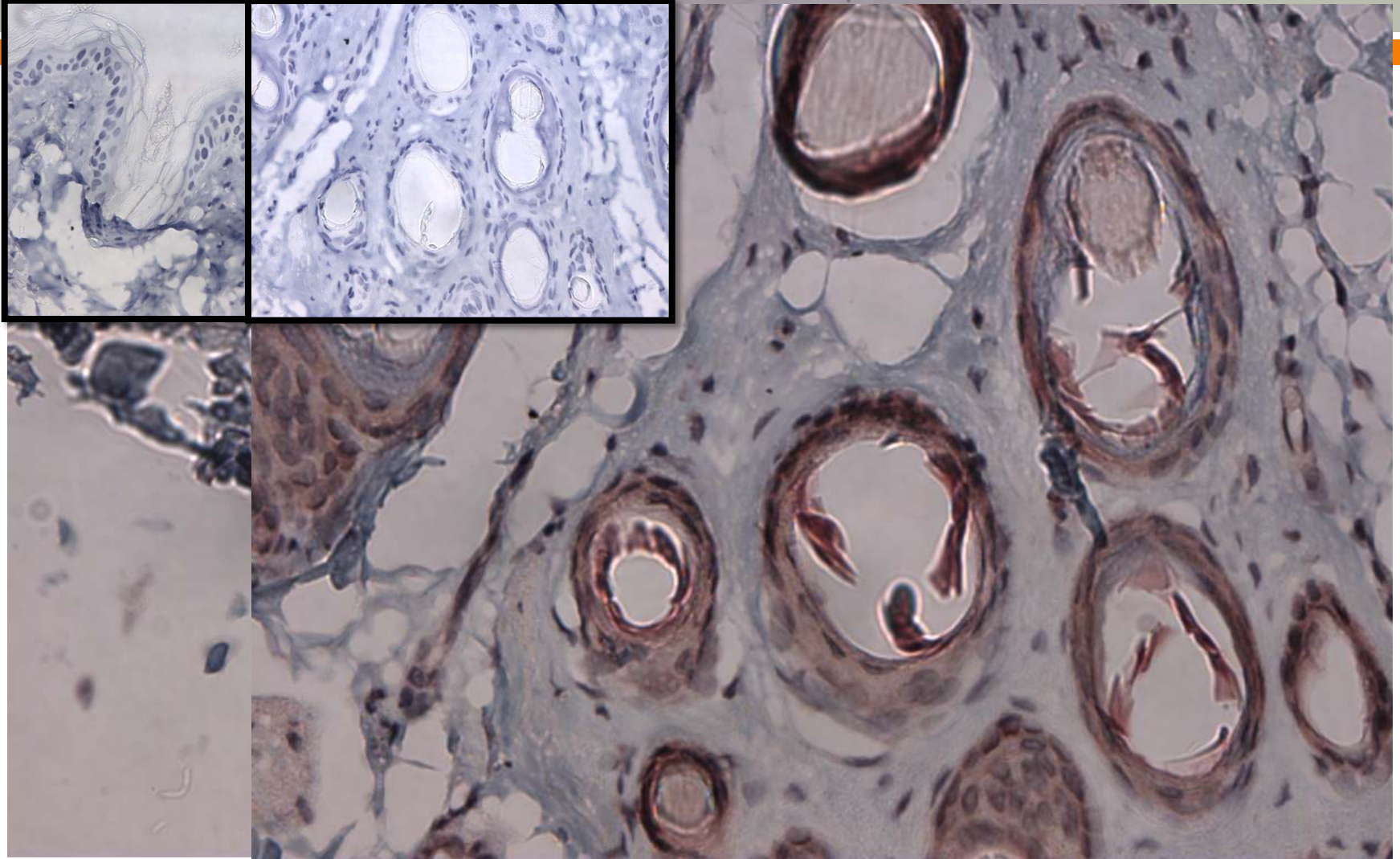
Suprelorin® F
(DESLORELIN ACETATE)
4.7mg IMPLANT

CAUTION: Federal law restricts this drug to use by or on the order of a licensed veterinarian.

Box contains 2 implants pre-loaded in sterile implanting needles and 1 non-sterile actuator syringe.

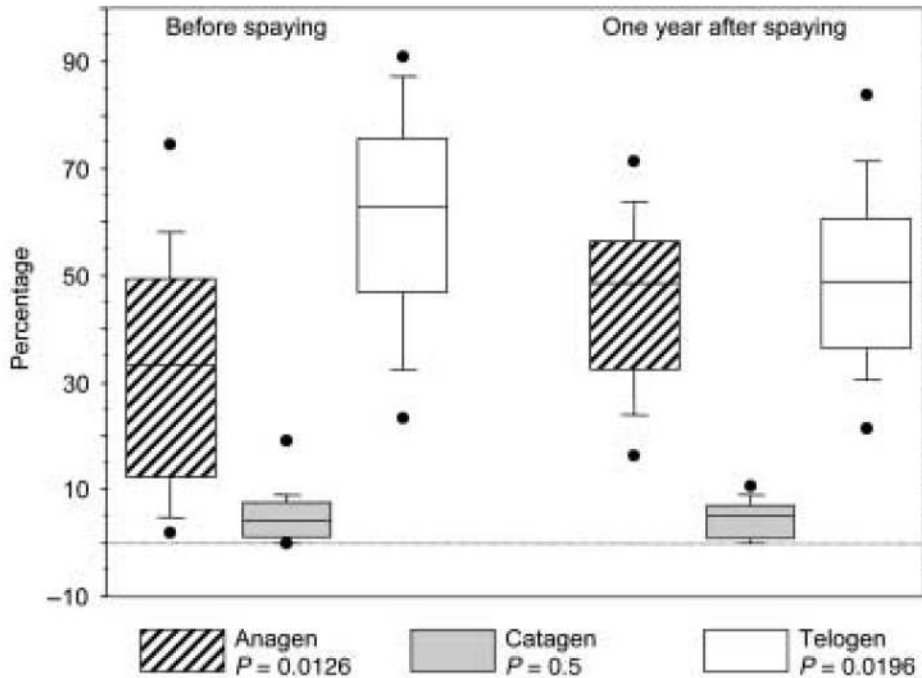
Belanger et al., 2017

Skin & Hair Follicles



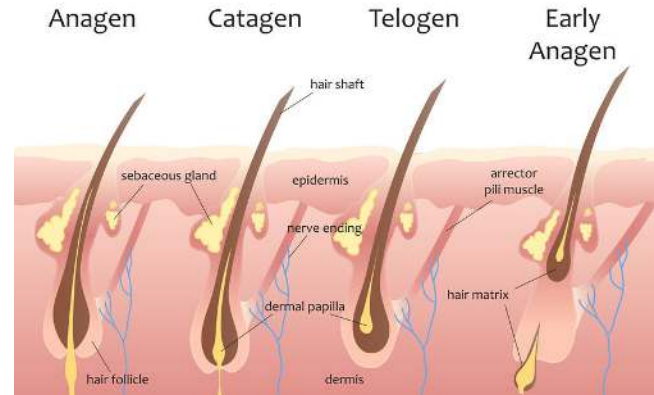
Puppy Coat Syndrome

Spaying results in coat changes in 20% of bitches



Reichler et al., 2008

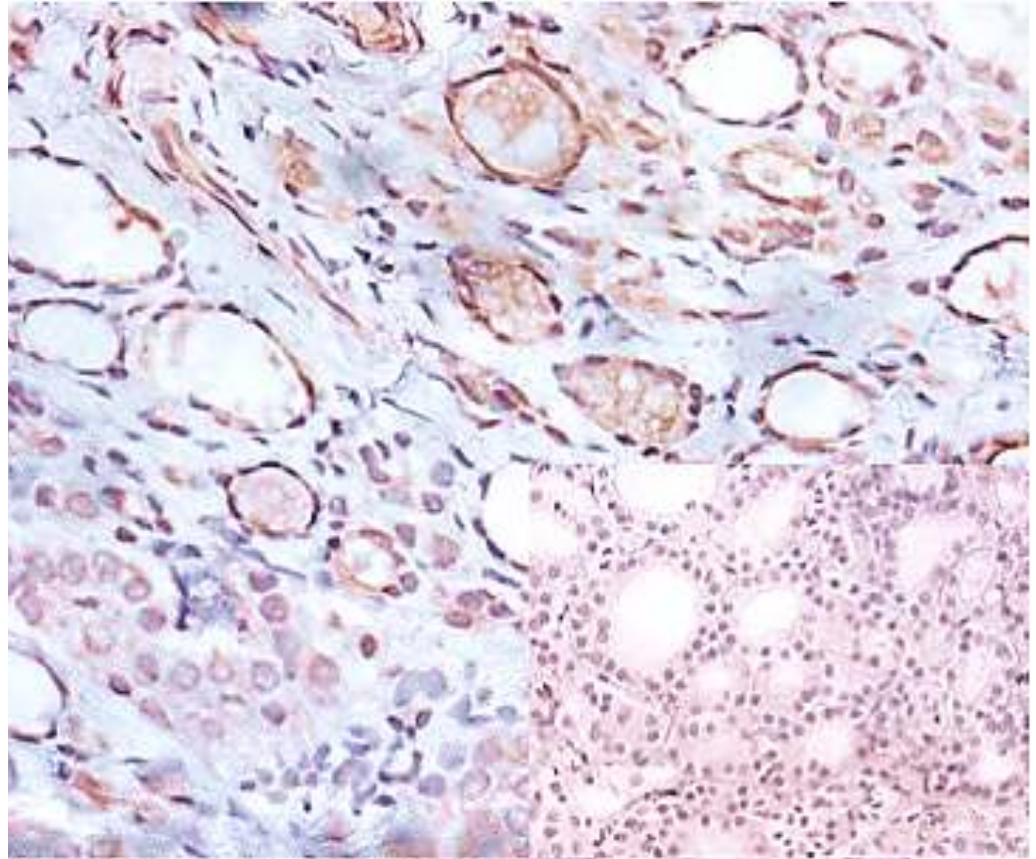
GnRH treatment results in temporary improvement of coat changes



Thyroid

∞ LH receptors are present in human & canine thyroid glands

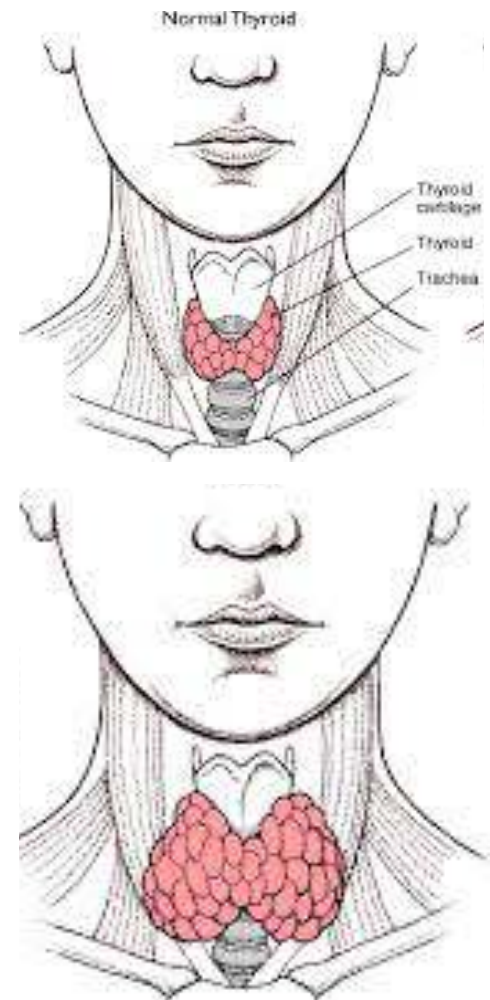
- Liu et al., 2014; Zwida & Kutzler, 2016



Percentage of positive cellular expression of $46.5 \pm 23.8\%$

Hypothyroidism in Humans

- Serum LH level is significantly greater in patients with thyroid adenoma
 - Liu et al., 2014
- Women who have undergone gonadectomy are also at an increased risk for developing hypothyroidism
 - De Leo et al., 1993
- Incidence of hypothyroidism is 10-15% higher in postmenopausal women
 - Giri et al., 2014



Hypothyroidism in Dogs

- Gonadectomy has a profound negative

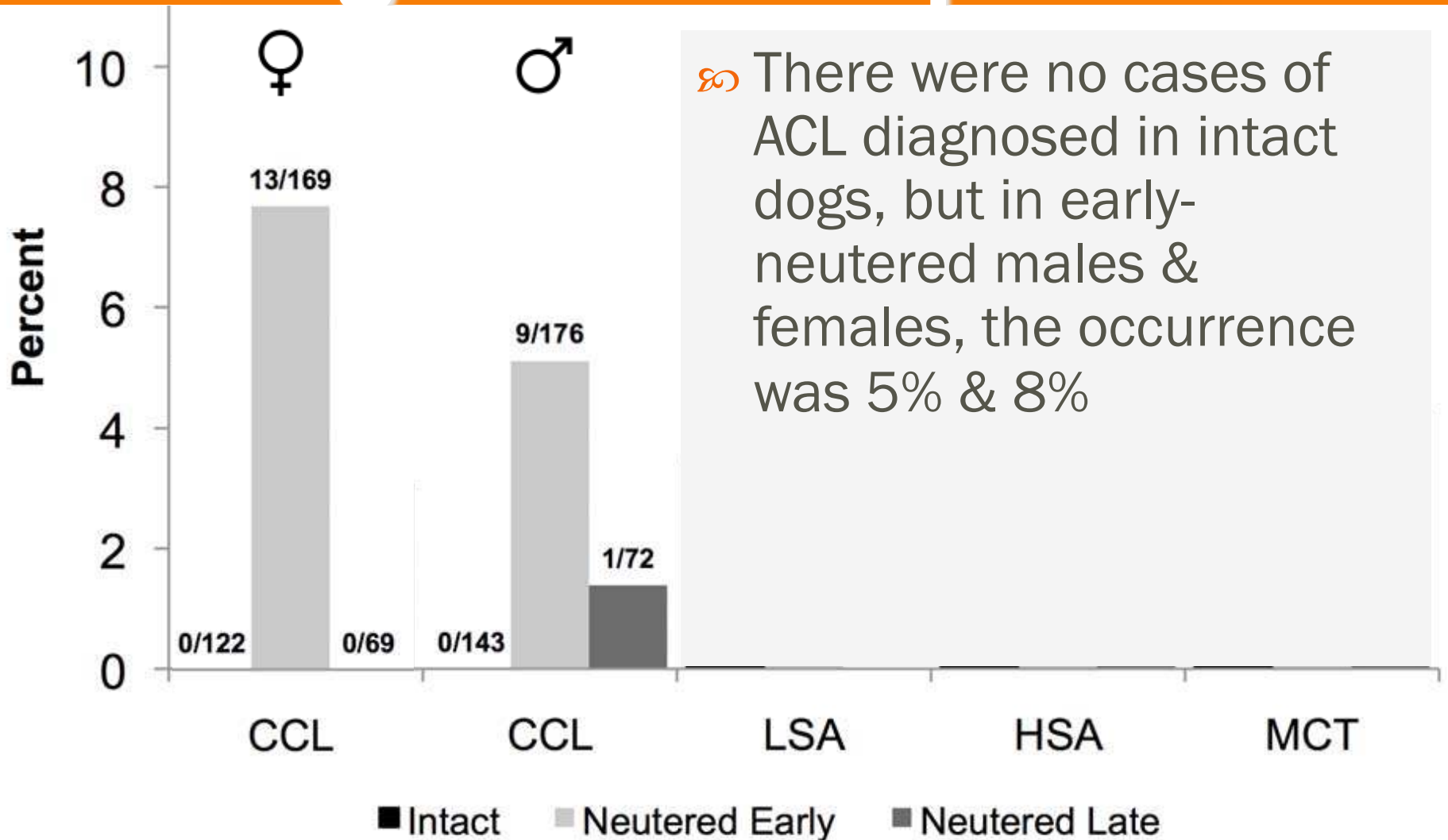


Table 3 Odds ratios (OR) and relative risk (RR) (\pm standard error) for the neutered female and male being more likely to express the condition (NA is not applicable)

Disease	OR		RR	
	Neutered female	Neutered male	Neutered female	Neutered male
Atopic Dermatitis (A/D)	2.24 \pm 0.27*	1.51 \pm 0.27*	2.21 \pm 0.26*	1.50 \pm 0.13*
Autoimmune Hemolytic Anemia (AHA)	1.67 \pm 0.28*	1.76 \pm 0.31*	1.67 \pm 0.28*	1.76 \pm 0.31*
Canine Myasthenia Gravis (CMG)	1.19 \pm 0.37	1.97 \pm 1.01	1.19 \pm 0.37	1.97 \pm 1.01
Colitis (COL)	1.03 \pm 0.11	0.98 \pm 0.09	1.03 \pm 0.11	0.98 \pm 0.08
Hypoadrenocorticism (ADC)	1.49 \pm 0.32*	2.07 \pm 0.54*	1.49 \pm 0.32*	2.07 \pm 0.53*
Hypothyroidism (HYPO)	3.03 \pm 0.39*	1.29 \pm 0.11*	2.99 \pm 0.39*	1.28 \pm 0.10*
Immune-Mediated Polyarthritis (IMPA)	1.49 \pm 0.37	1.02 \pm 0.14	1.49 \pm 0.37	1.02 \pm 0.14
Immune-Mediated Thrombocytopenia (ITP)	3.14 \pm 0.73*	2.05 \pm 0.42*	3.13 \pm 0.73*	2.05 \pm 0.42*
Inflammatory Bowel Disease (IBD)	2.20 \pm 0.54*	1.43 \pm 0.23*	2.19 \pm 0.54*	1.43 \pm 0.23*
Lupus Erythematosus (LUP)	2.64 \pm 1.24*	0.68 \pm 0.16	2.64 \pm 1.24*	0.68 \pm 0.16
Pemphigus Complex (PEMC)	1.35 \pm 0.39	1.64 \pm 0.56	1.35 \pm 0.39	1.64 \pm 0.56
Pyometra (PYO)	0.04 \pm 0.01*	NA	0.04 \pm 0.01*	NA

Asterisks indicate a significant difference from the intact counterpart ($p < 0.05$)

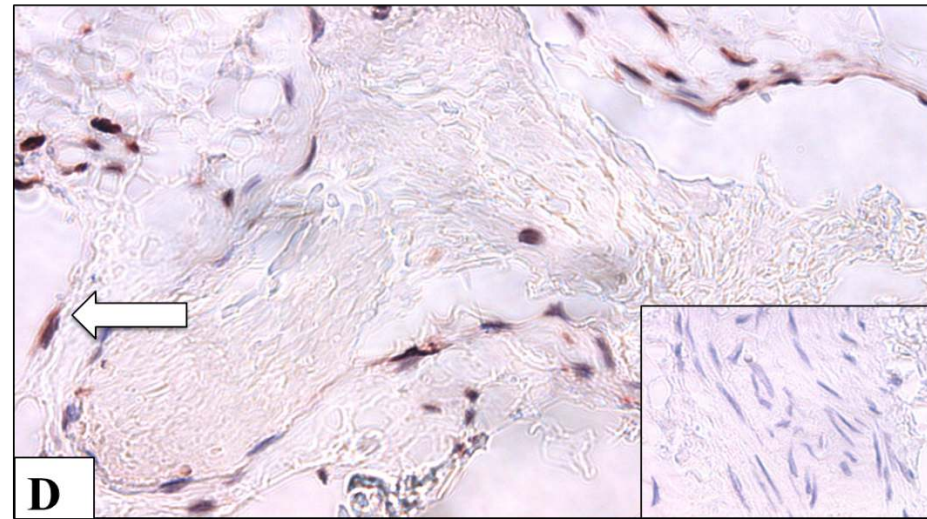
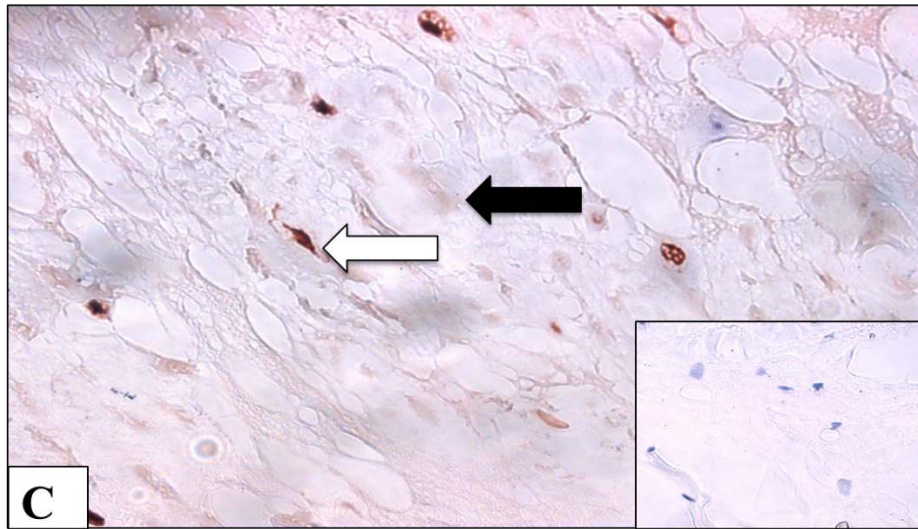
Anterior Cruciate Ligament Rupture



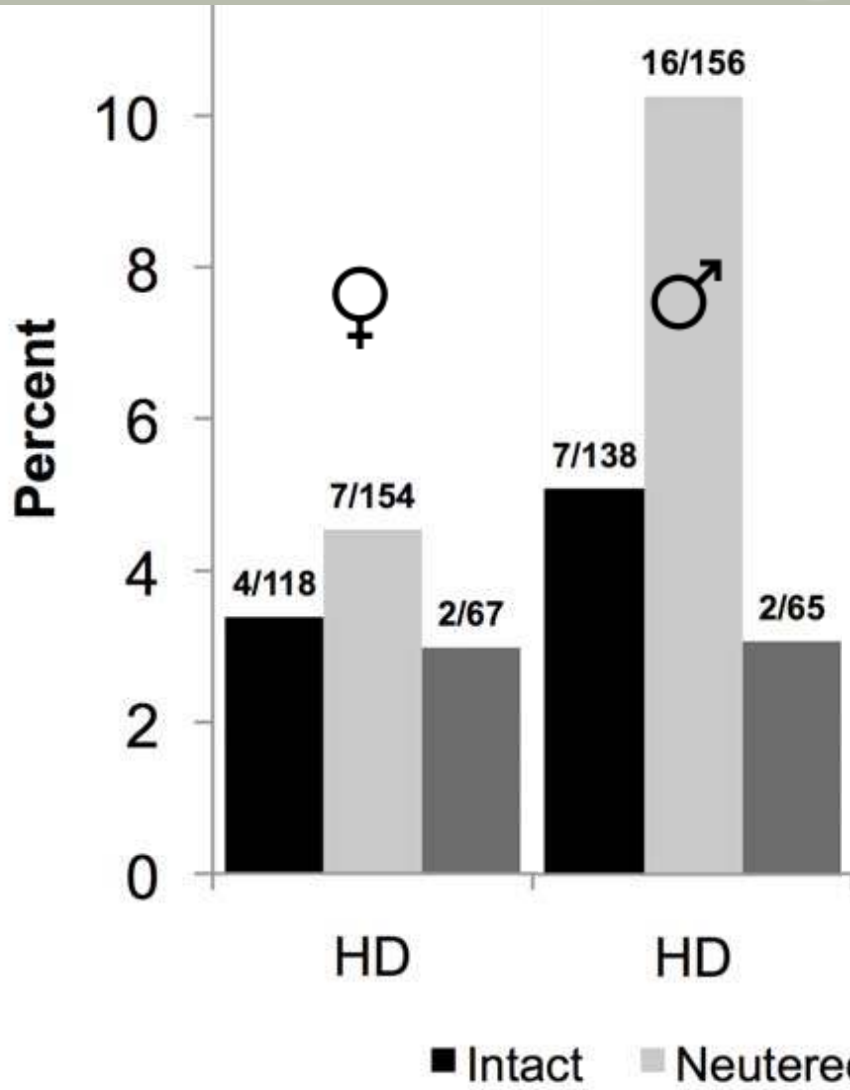
Anterior Cruciate Ligament Rupture

∞ Our laboratory has demonstrated the expression of LH receptors within the anterior cruciate ligament & synovia

- Kiefel et al., 2016; Kiefel & Kutzler, 2018



Hip Dysplasia

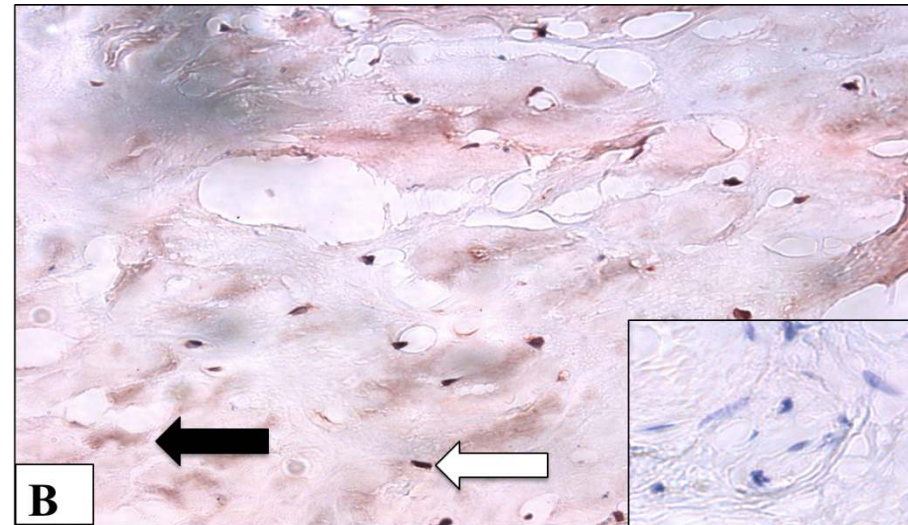
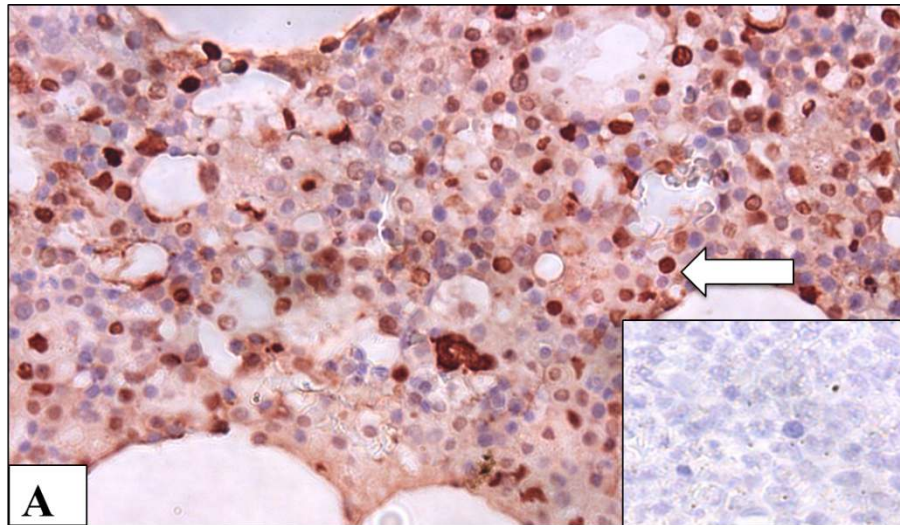


- Of early-neutered males, 10% were diagnosed with HD
 - Twice the occurrence in intact males

Hip Dysplasia

∞ Our laboratory has demonstrated the expression of LH receptors within the femoral subchondral bone & round ligament

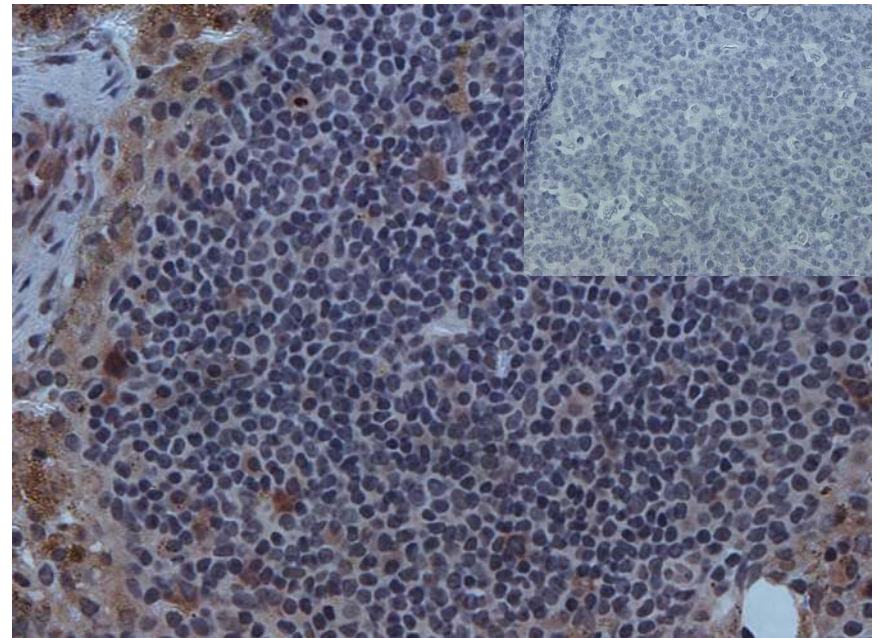
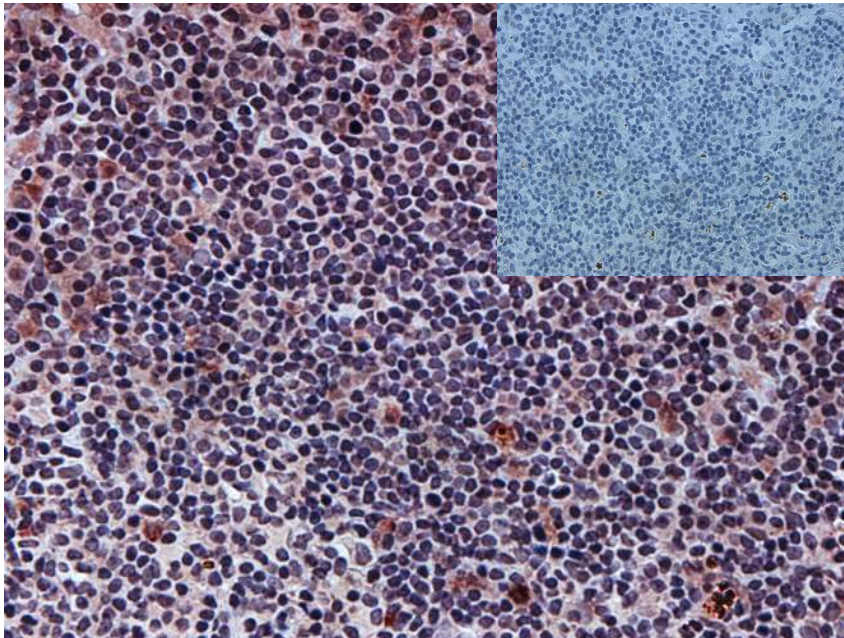
- Kiefel et al, 2016; Kiefel & Kutzler, 2018



Lymphocytes

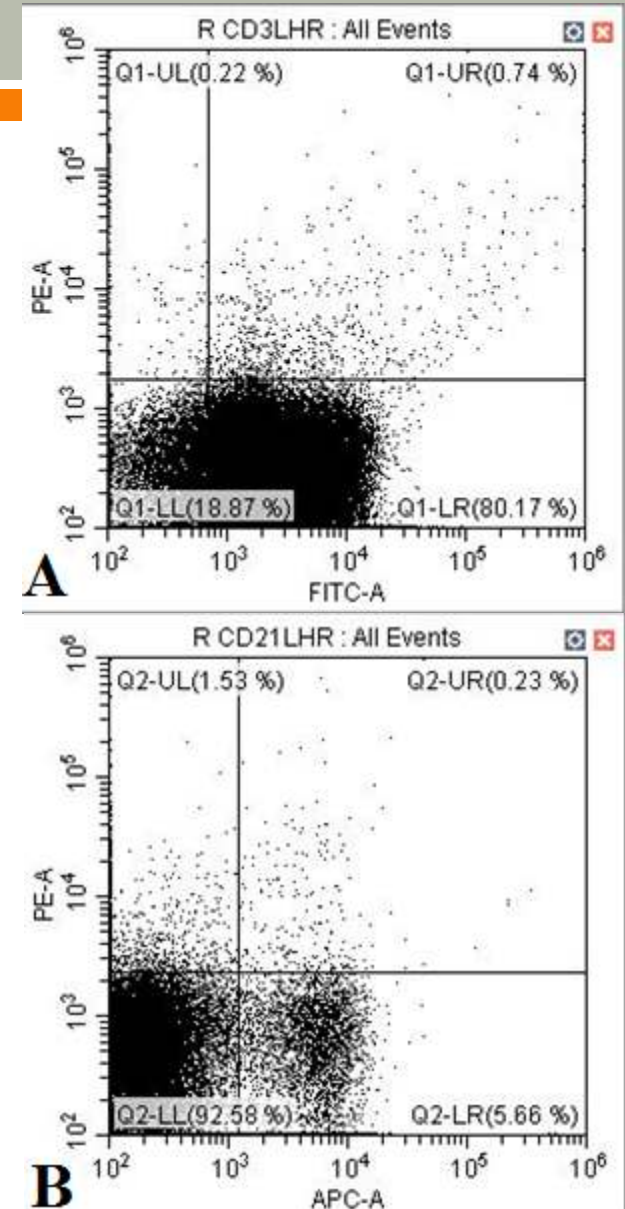
∞ Canine lymphocytes express LHR in 4% of cells from normal lymph nodes

- Ettinger & Kutzler 2017



Lymphocytes

- 🌀 All dogs tested expressed LHR in circulating B- & T-lymphocytes
 - Trend for increased LHR expression in circulating B-lymphocytes from male dogs ($19.65 \pm 13.53\%$) compared to female dogs ($9.61 \pm 5.35\%$; $p=0.06$) but not in T-lymphocytes
 - LHR expression varied by sex status in circulating T-lymphocytes with spayed and neutered dog having higher LHR expression ($16.58 \pm 7.81\%$) compared to intact dogs ($10.53 \pm 2.31\%$; $p=0.049$)



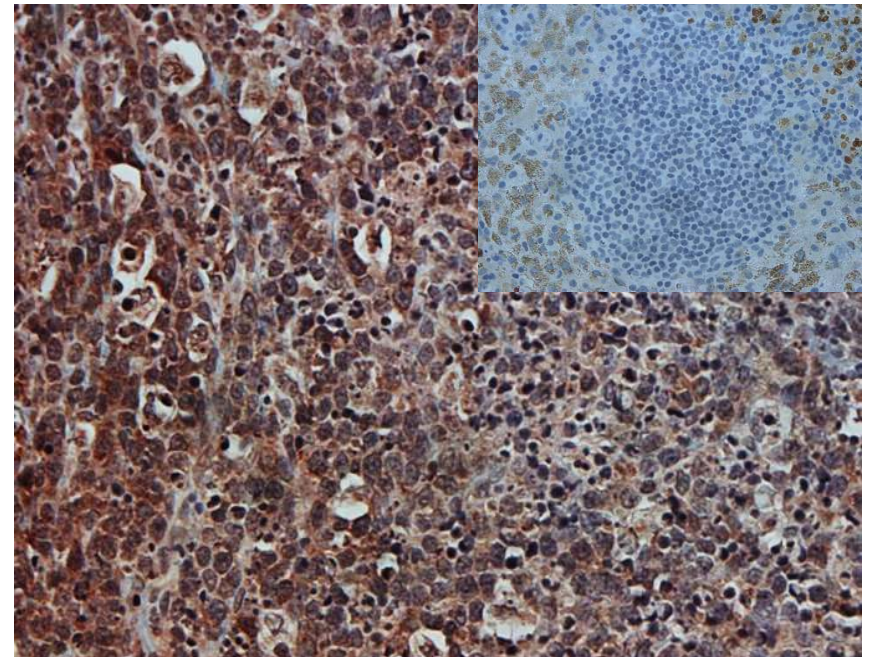
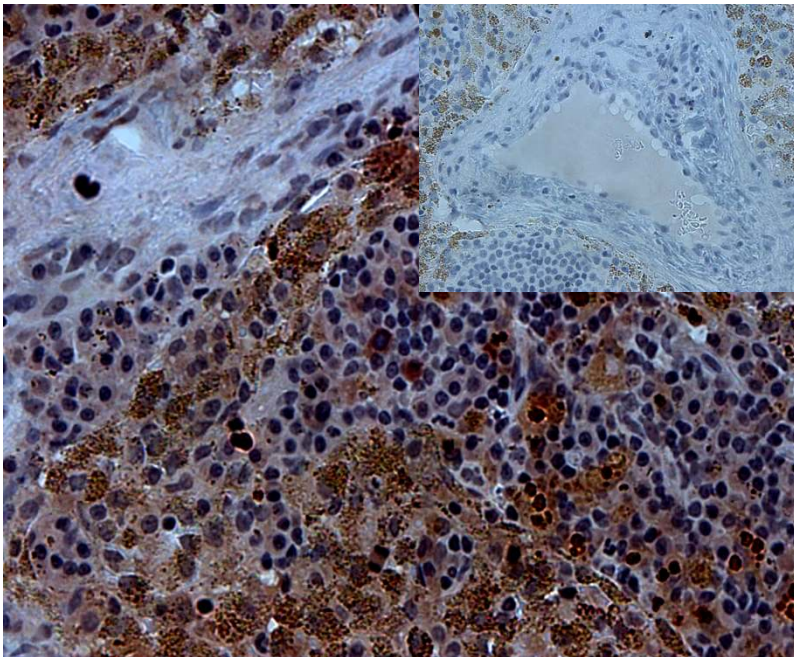
Lymphoma

- ∞ Cancer of lymphocytes &/or lymphoid tissues
- ∞ Most common cancer diagnosed in dogs accounting for up to 24% of all canine cancers
 - Vail et al., 2001
- ∞ Gonadectomy increases the incidence of lymphosarcoma
 - Zink et al., 2014
 - Gonadectomized males are three times more likely to develop lymphosarcoma than unaltered males and about 1 in 10 neutered males will develop lymphosarcoma
 - Torres et al, 2013

Lymphoma

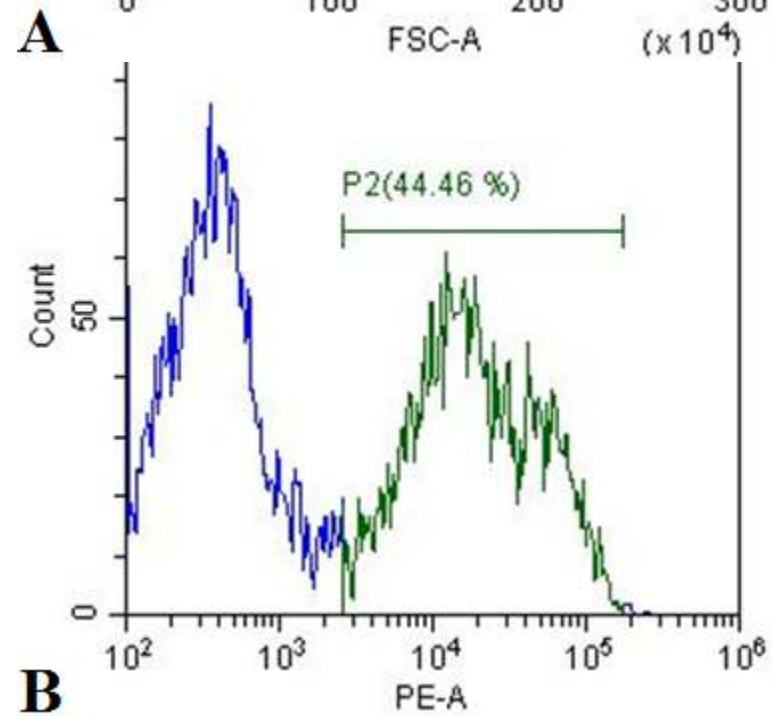
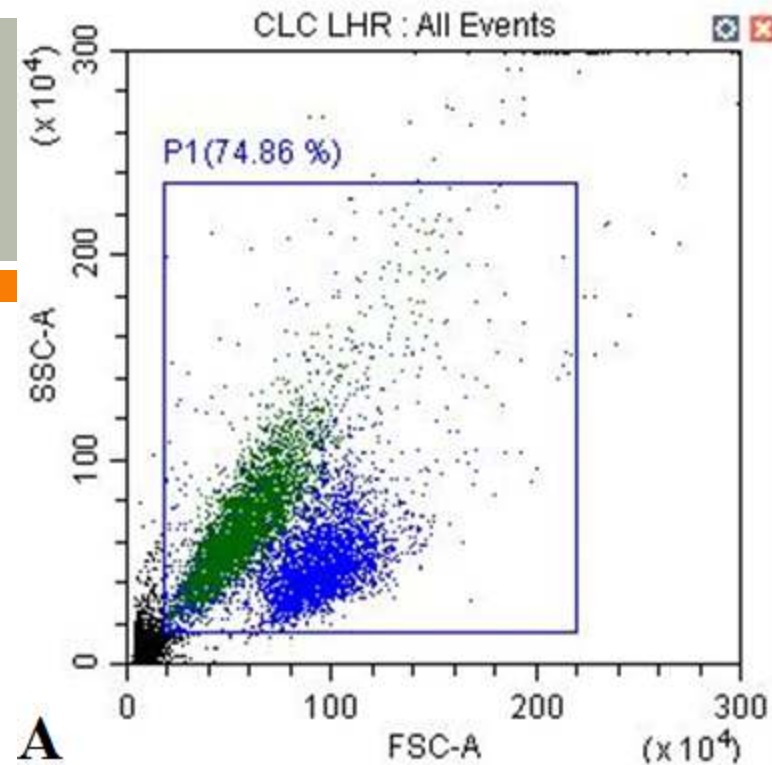
∞ Canine lymphocytes express LHR in 12.37% of lymphocytes from neoplastic lymph nodes

- Ettinger & Kutzler 2017



Lymphoma

- ∞ Cultured neoplastic T-lymphocytes isolated from three dogs with lymphoma also expressed LHR albeit to a different level of expression in each cell line, with either 45%, 35% or 10% of cells expressing LHR
 - In all of the cell lines, the cell population that expressed LHR was smaller in size (forward scatter) and more granular (side scatter)



Mastocytoma

☞ Most common skin tumor in dogs

- Shoop et al., 2015

☞ LH receptors are abundant in mastocytoma cells

- Moccia & Kutzler, 2018

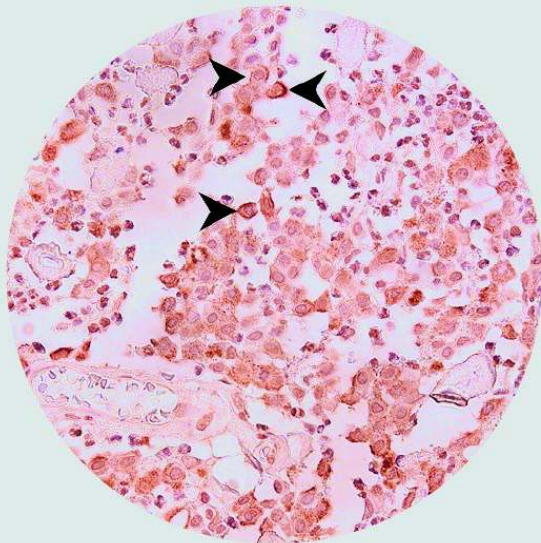


Figure 1. LHR- staining pattern I is characterized by membrane-associated staining (black arrowheads), with little cytoplasmic staining of neoplastic mast cells (black arrowheads).

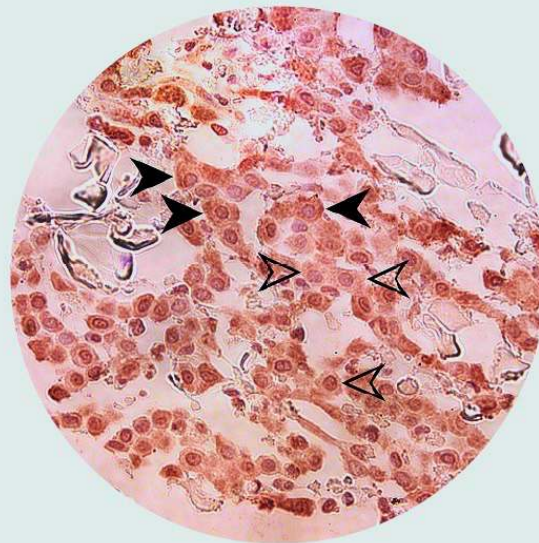
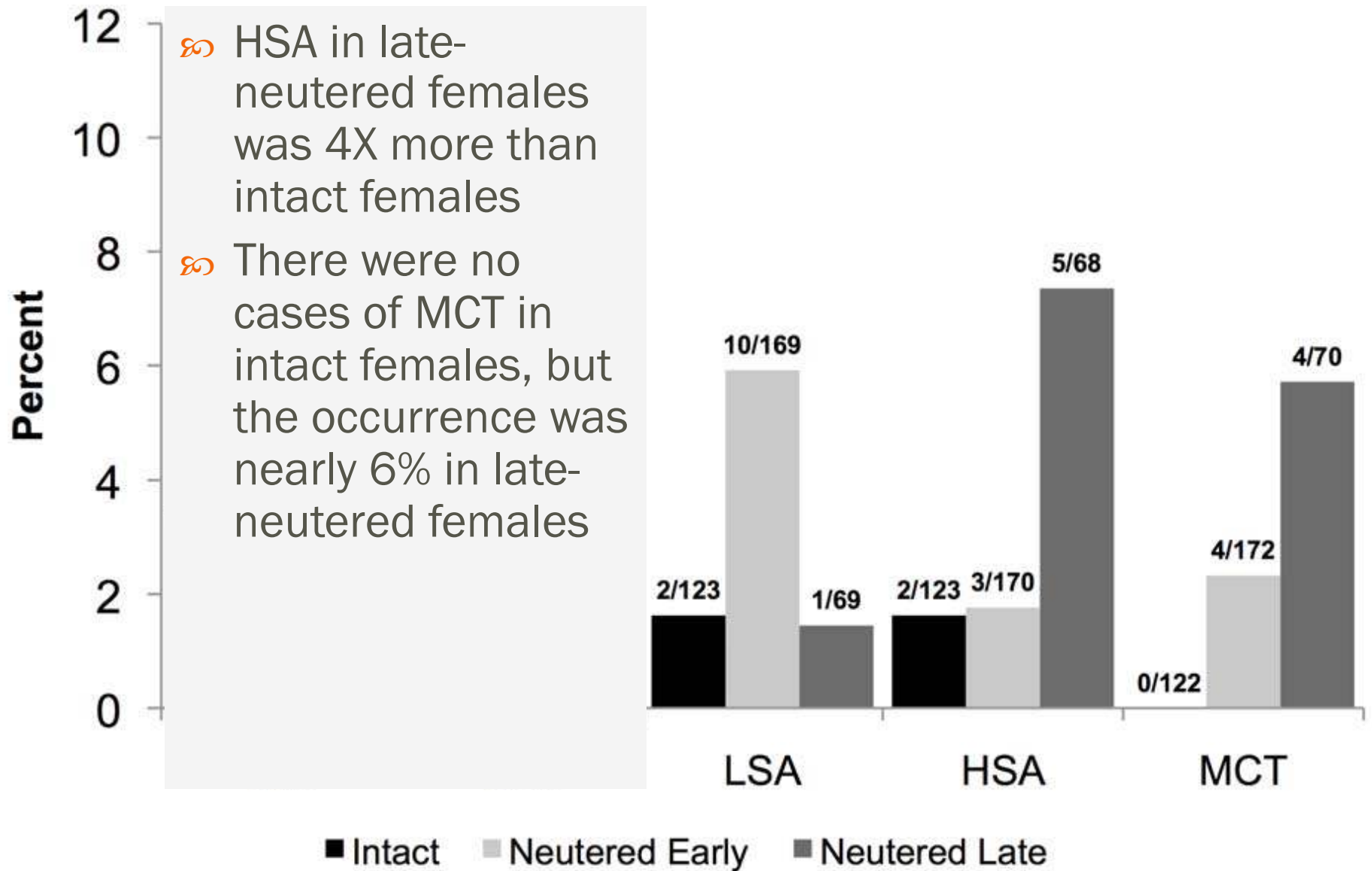


Figure 2. LHR- staining pattern II is characterized by low (open arrowheads) to medium (black arrowheads) cytoplasmic intensity staining with small granules in neoplastic mast cells.



Figure 3. LHR- staining pattern III is characterized by high staining intensity (black arrowheads). Some neoplastic mast cells have staining of coalesced granules.

Female Golden Retrievers



Evaluation of the risk and age of onset of cancer and behavioral disorders in gonadectomized Vizslas

M. Christine Zink, DVM, PhD; Parvene Farhooody, MA; Samra E. Elser, MS; Lynda D. Ruffini; Tom A. Gibbons, MS; Randall H. Rieger, PhD



Objective—To investigate associations between age at gonadectomy and estimated risk or age at diagnosis of neoplastic and behavioral disorders in Vizslas.

Design—Retrospective cohort study.

Animals—2,505 Vizslas born between 1992 and 2008.

Procedures—Data on demographics, gonadectomy status, and age at diagnosis of disease or disorder were obtained with an anonymous online survey and analyzed.

Results—Dogs gonadectomized at ≤ 6 months, between 7 and 12 months, or at > 12 months of age had significantly increased odds of developing mast cell cancer, lymphoma, all other cancers, all cancers combined, and fear of storms, compared with the odds for sexually intact dogs. Females gonadectomized at ≤ 12 months of age and males and females gonadectomized at > 12 months of age had significantly increased odds of developing hemangiosarcoma, compared with the odds for sexually intact dogs. Dogs gonadectomized at ≤ 6 months of age had significantly increased odds of developing a behavioral disorder. The younger the age at gonadectomy, the earlier the mean age at diagnosis of mast cell cancer, cancers other than mast cell, hemangiosarcoma, lymphoma, all cancers combined, a behavioral disorder, or fear of storms.

Conclusions and Clinical Relevance—Additional studies are needed on the biological effects of removing gonadal hormones and on methods to render dogs infertile that do not involve gonadectomy. Veterinarians should discuss the benefits and possible adverse effects of gonadectomy with clients, giving consideration to the breed of dog, the owner's circumstances, and the anticipated use of the dog. (*J Am Vet Med Assoc* 2014;244:309–319)



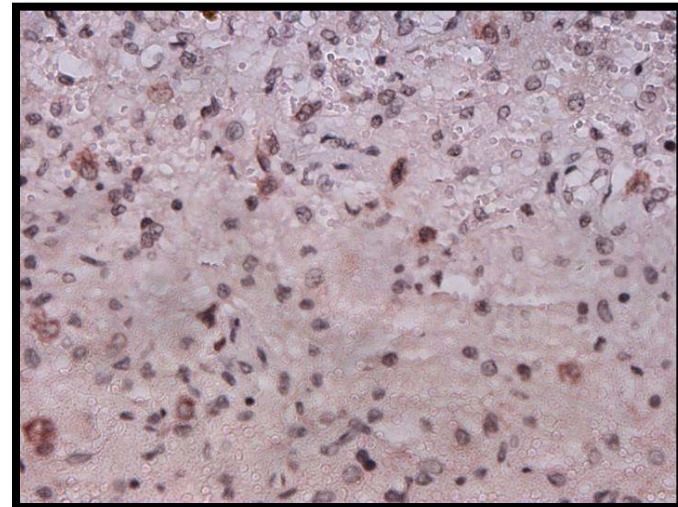
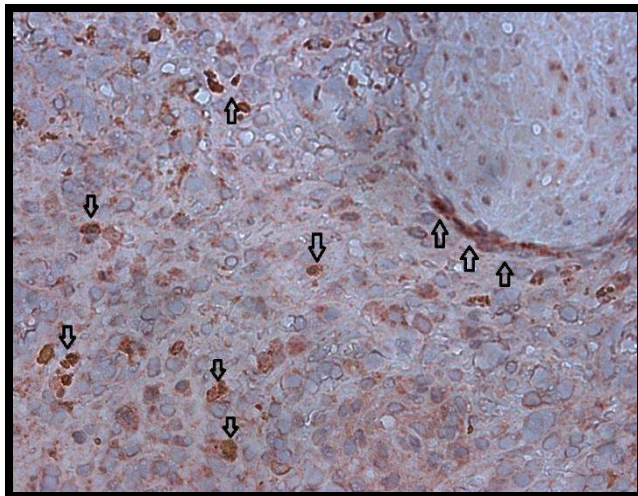
Hemangiosarcoma

∞ Many studies have confirmed the presence of LH receptors in vascular endothelial and smooth muscle cells

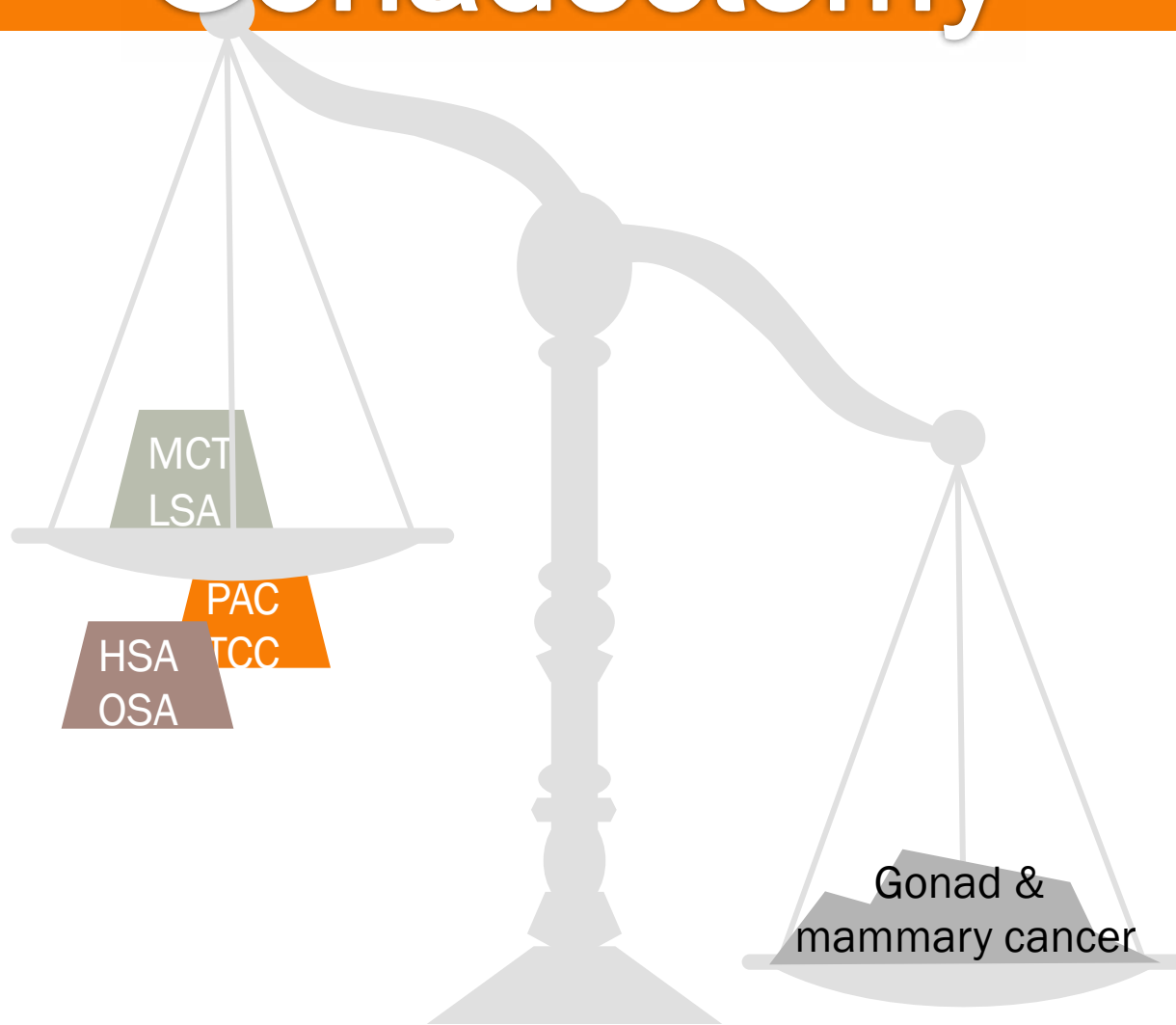
- Lei et al., 1993; Reshef et al., 1990

∞ Our laboratory has demonstrated the expression of LH receptors in splenic hemangiosarcomas

- Zwida et al 2017



Risk-Benefits of Gonadectomy



~ Closing Remarks ~

- ❧ Traditional ovariohysterectomy (spay) & neuter still has its place in veterinary medicine but dog owners should be aware of the physiologic implications of removing the gonads & then make an educated decision about the lifetime health of their pet

Acknowledgements

- ∞ Dr. David Waters
- ∞ Dr. Benjamin Hart & Lynette Hart
- ∞ Dr. Marty Becker
- ∞ Khawla Zwida, Alyssa Ettinger, Sabrina Gust, Camryn Flint, Alyssa Vedus, Claire Kiefel, Valerio Moccia, Karina Hallam,
- ∞ Parsemus Foundation & Elaine Lissner

Any Questions ??



**HELP CONTROL THE
HUMAN POPULATION**

Have your human spayed or neutered

