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

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With gonads

Hypothalamus
GnRH

Pituitary Gland
LH

Gonads
E2/T2

Without gonads

Hypothalamus
GnRH

Pituitary Gland
LH

LH is up to 30X higher

No Negative feedback
When LH binds to its receptor, it induces cell division & stimulates nitric oxide release

- Greene & Ginther, 2011
## LHR in Non-Reproductive Tissues

<table>
<thead>
<tr>
<th>Non-Reproductive Tissues</th>
<th>Species</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal cortex</td>
<td>Dog, human, rat, rhesus macaque</td>
<td>Papadopoulos et al., 1991; Galac et al., 2010; Nicolini et al., 2014; Lasley et al., 2015</td>
</tr>
<tr>
<td>Blood vessels (endothelial cells, vascular smooth muscle cells)</td>
<td>Human</td>
<td>Reshef et al., 1990; Lei et al., 1993; Bukovsky et al., 2003</td>
</tr>
<tr>
<td>Brain (hippocampus, hypothalamus, cerebellum, brain stem, cortex)</td>
<td>Guinea pig, rat</td>
<td>Lei et al., 1993; Wahjoepramono et al., 2011</td>
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<tr>
<td>Fibroblasts</td>
<td>Human</td>
<td>Bukovsky et al., 2003</td>
</tr>
<tr>
<td>Gastrointestinal tract (enteric neurons, smooth muscle)</td>
<td>Human, rat</td>
<td>Hammar et al., 2012; Sand et al., 2013, Ducker et al., 1996</td>
</tr>
<tr>
<td>Lower urinary tract (bladder and urethra)</td>
<td>Dog, human</td>
<td>Welle et al., 1999; Ponglowhapan et al., 2007; Ponglowhapan et al., 2008; Schwalenberg et al., 2012</td>
</tr>
<tr>
<td>Lymphoid tissues (thymus and lymphocytes)</td>
<td>Hamsters, human</td>
<td>Maria, 1998; Seiki et al., 1990</td>
</tr>
<tr>
<td>Skin (epidermis, hair follicle, sebaceous glands, sweat glands)</td>
<td>Dog, human</td>
<td>Welle et al., 2006; Venencie et al., 1999</td>
</tr>
<tr>
<td>Striated muscle cells</td>
<td>Human</td>
<td>Bukovsky et al., 2003</td>
</tr>
<tr>
<td>Thyroid gland</td>
<td>Human</td>
<td>Liu et al., 2014</td>
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</table>
LH receptors are expressed in all regions of the canine lower urinary tract (body & neck of the bladder, proximal & distal urethra) & in all tissue layers (epithelium, sub-epithelial stroma & muscle)

- Ponglowapan 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
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
Gonadectomized female dogs with urinary incontinence have a significantly higher number of LH receptors in the lower urinary tract compared to unaltered females.

- Coit 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)
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

<table>
<thead>
<tr>
<th>Disease</th>
<th>Neutered Female</th>
<th>Neutered Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ocular</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early onset Cataracts</td>
<td>0.42 ± 0.04</td>
<td>0.66 ± 0.07</td>
</tr>
<tr>
<td>Lens luxation</td>
<td>1.13 ± 0.17</td>
<td>1.19 ± 0.20</td>
</tr>
<tr>
<td>Orthopedic</td>
<td></td>
<td></td>
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<tr>
<td>Elbow dysplasia</td>
<td>0.91 ± 0.09</td>
<td>0.89 ± 0.07</td>
</tr>
<tr>
<td>Hip Dysplasia</td>
<td>0.93 ± 0.07</td>
<td>0.96 ± 0.06</td>
</tr>
<tr>
<td>Intervertebral disk disease</td>
<td>1.70 ± 0.10</td>
<td>1.06 ± 0.04</td>
</tr>
<tr>
<td>Patellar luxation</td>
<td>0.99 ± 0.06</td>
<td>0.95 ± 0.06</td>
</tr>
<tr>
<td>Ruptured Anterior Cruciate Ligament</td>
<td>3.18 ± 0.45</td>
<td>2.32 ± 0.28</td>
</tr>
<tr>
<td>Cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemangiosarcoma</td>
<td>3.18 ± 0.73</td>
<td>1.39 ± 0.17</td>
</tr>
<tr>
<td>Hyperadrenocorticism (cushings)</td>
<td>4.56 ± 0.76</td>
<td>2.02 ± 0.24</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>2.25 ± 0.28</td>
<td>1.20 ± 0.09</td>
</tr>
<tr>
<td>Mast cell tumor</td>
<td>2.78 ± 0.33</td>
<td>1.25 ± 0.11</td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>2.53 ± 0.47</td>
<td>1.62 ± 0.20</td>
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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
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 effect on thyroid function & is the most significant cause for the development of hypothyroidism in dogs
  - Dixon & Mooney, 1999; Panciera, 1984
  - 30% more gonadectomized dogs develop hypothyroidism compared to unaltered dogs
    - Milne & Hayes, 1981
  - T4 concentration in gonadectomized dogs is significantly lower when compared to intact dogs
    - Modawska et al., 2014; Günzel-Apel et al., 2009

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

<table>
<thead>
<tr>
<th>Disease</th>
<th>OR</th>
<th>RR</th>
</tr>
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<tbody>
<tr>
<td>Atopic Dermatitis (ATOP)</td>
<td>2.24 ± 0.27*</td>
<td>2.21 ± 0.26*</td>
</tr>
<tr>
<td>Autoimmune Hemolytic Anemia (AIHA)</td>
<td>1.67 ± 0.28*</td>
<td>1.67 ± 0.28*</td>
</tr>
<tr>
<td>Canine Myasthenia Gravis (CMG)</td>
<td>1.19 ± 0.37</td>
<td>1.19 ± 0.37</td>
</tr>
<tr>
<td>Colitis (COL)</td>
<td>1.03 ± 0.11</td>
<td>1.03 ± 0.11</td>
</tr>
<tr>
<td>Hypoadrenocorticism (ADO)</td>
<td>1.49 ± 0.32*</td>
<td>1.49 ± 0.32*</td>
</tr>
<tr>
<td>Hypothyroidism (HYPO)</td>
<td>3.03 ± 0.39*</td>
<td>2.99 ± 0.39*</td>
</tr>
<tr>
<td>Immune-Mediated Polyarthritis (IMPA)</td>
<td>1.49 ± 0.37</td>
<td>1.49 ± 0.37</td>
</tr>
<tr>
<td>Immune-Mediated Thrombocytopenia (ITP)</td>
<td>3.14 ± 0.73*</td>
<td>3.13 ± 0.73*</td>
</tr>
<tr>
<td>Inflammatory Bowel Disease (IBD)</td>
<td>2.20 ± 0.54*</td>
<td>2.19 ± 0.54*</td>
</tr>
<tr>
<td>Lupus Erythematosus (LUP)</td>
<td>2.64 ± 1.24*</td>
<td>2.64 ± 1.24*</td>
</tr>
<tr>
<td>Pemphigus Complex (PEMC)</td>
<td>1.35 ± 0.39</td>
<td>1.35 ± 0.39</td>
</tr>
<tr>
<td>Pyometra (PYO)</td>
<td>0.04 ± 0.01*</td>
<td>0.04 ± 0.01*</td>
</tr>
</tbody>
</table>

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

Sundburg et al., 2016
Anterior Cruciate Ligament Rupture

There were no cases of ACL diagnosed in intact dogs, but in early-neutered males & females, the occurrence was 5% & 8%.
Our laboratory has demonstrated the expression of LH receptors within the anterior cruciate ligament & synovia

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

- Independent of obesity, gonadectomy significantly increases the incidence of hip dysplasia


- Compared to unaltered dogs, gonadectomy increases the incidence by 1.5-2X the occurrence in unaltered dogs


- Of early-neutered males, 10% were diagnosed with HD
  - Twice the occurrence in intact males
Our laboratory has demonstrated the expression of LH receptors within the femoral subchondral bone & round ligament

- Kiefel et al, 2016; Kiefel & Kutzler, 2018
Canine lymphocytes express LHR in 4% of cells from normal lymph nodes

- Ettinger & Kutzler 2017
All dogs tested expressed LHR in circulating B- & T-lymphocytes

- Trend for increased LHR expression in circulating B-lymphocytes from male dogs (19.65±13.53%) compared to female dogs (9.61±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±7.81%) compared to intact dogs (10.53±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
Canine lymphocytes express LHR in 12.37% of lymphocytes from neoplastic lymph nodes

- Ettinger & Kutzler 2017
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.
Almost 10% of early-neutered males developed LSA (3X more than intact males).

HSA in late-neutered females was 4X more than intact females.

There were no cases of MCT in intact females, but the occurrence was nearly 6% in late-neutered females.
Evaluation of the risk and age of onset of cancer and behavioral disorders in gonadectomized Vizslas

M. Christine Zink, dvm, phd; Parvene Farhoody, ma; Samra E. Elser, bs; 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.


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)
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

MCT
LSA
PAC
TCC
HSA
OSA

Gonad & mammary cancer
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

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Parsemus Foundation & Elaine Lissner
Any Questions ??

HELP CONTROL THE HUMAN POPULATION
Have your human spayed or neutered