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The correlation of neuter status and clinical manifestations of orthopaedic disorders- systematic literature review

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Budapest, Hungary

2021

ABSTRACT

Elective gonadectomy is a widespread surgery for dogs and has many positive effects on health, population control, behaviour and longevity. Adverse effects include a predisposition to some neoplasia's, cognitive decline, and the development of orthopaedic conditions. There are also adverse effects associated with neutering that depend upon the age of neutering, sex and breed.

This systematic literature review aims to analyse the correlation between neuter status and clinical manifestation of specific orthopaedic disorders in dogs and objectively evaluate the strength of evidence for the association. This paper will first examine the benefits and risks of gonadectomy before focusing on the specific role of the sex hormones in the orthopaedic system. Then six specific orthopaedic conditions will be examined in detail. These include Cranial Cruciate Ligament Rupture (CCLR), Hip Dysplasia (HD), Elbow Dysplasia (ED), Patellar Luxation, Intervertebral Disc Disease (IVDD) and Osteosarcoma.

To evaluate if neutering, the age of neutering, and the sex of the dog influence the onset of these conditions, three categories of studies were used. These included studies that used data from Veterinary Medical Databases, questionnaire-based studies and experimental studies. All the studies used in this literature review had a large population size so that the results were statistically accurate, and the articles were written by respected authors. In all studies chosen for the literature review, predisposing factors that could have influenced the development of these orthopaedic conditions, such as obesity, were taken into consideration.

The results of this literature review found that neutering did increase the prevalence of these specific orthopaedic conditions. It was observed that early neutering of dogs significantly increased the risk of the occurrence of these conditions and that dogs neutered after 12 months of age had a significantly decreased risk compared to those neutered before 12 months of age. Whether male or female dogs were more affected was variable and depended on each specific condition.

Regarding the six specific orthopaedic conditions, it was seen that the occurrence of CCLR increased with early neutering in both sexes, and the occurrence of this condition in intact dogs was negligible. The occurrence of HD was shown to increase in neutered dogs, specifically in early neutered dogs. It was also shown that male dogs were significantly more at risk of this condition than female dogs. ED was shown to have an increased prevalence in

early neutered dogs, specifically in male dogs. Patellar luxation was seen to have an increased prevalence in female dogs compared to male dogs, and early neutering of both sexes increased the occurrence of this condition. Concerning IVDD, it was seen that neutering was associated with a greater prevalence of the condition in neutered compared to intact dogs in both sexes. However, in female dogs, neutering whether early or late resulted in a higher incidence of IVDD, in male dogs, only dogs that were neutered early had an increased susceptibility to developing IVDD. Neutering was shown to be a predisposing factor in the development of osteosarcomas in dogs of both sexes, and male dogs were shown to be marginally more susceptible to the condition than female dogs

This systematic literature review concluded that there is a proven correlation between neutering and the development of orthopaedic conditions and that this was influenced by the age at neutering and this by the sex of the dog. However, while in female dogs where the risks involved with leaving the dogs intact such as the development of mammary gland tumours and pyometra, outweigh the risk of the increased occurrence of these orthopaedic conditions. In male dogs, the medical benefits of neutering are negligible, and the risks associated with the procedure are significant. However neutering in male dogs is proven to reduce aggressive behaviour and plays a role in population control. It would be the recommendation of this paper that female dogs should be neutered routinely, but in male dogs, the risks outweigh the benefit of this procedure.

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ABBREVIATIONS

- E2- Estradiol
- LH- Lutenising Hormone
- FSH- Follicle Stimulating Hormone
- LHR- Lutenising Hormone Receptor
- FSHR- Follicle Stimulating Hormone Receptor
- HSA- Hemangiosarcoma
- GnRH- Gonadotropin- Releasing Hormone
- ER-α- Estrogen Receptor Alpha
- ER β - Estrogen Receptor Beta
- ECM- Extracellular Matrix
- ELC- Estrogen Like Compounds
- NF-κB- Nuclear Factor-κB Ligand
- ROS- Reactive Oxygen Species
- DHT- 5α -Dihydrotestosterone
- TGFb- Transforming Growth Factor Beta
- IGFs- Insulin Like Growth Factors
- IL-6- Interleukin 6
- RANKL- Receptor Activator of Nuclear Factor-κB Ligand
- BCS- Body Condition Score
- CCL- Cranial Cruciate Ligament
- HD- Hip Dysplasia
- ED- Elbow Dysplasia
- IVDD- Intervertebral Disc Disease
- IVDH- Intervertebral Disc Herniation

INTRODUCTION

Neutering has been a common practice in the canine world for decades. It was introduced for several reasons, particularly as an instrument of population control. While there are many benefits associated with removing the sexual gonads of canines, there are also disadvantages connected to this practice. The aim of this thesis is to address such drawbacks, specifically, the association of neutering with orthopaedic conditions. Firstly, this paper will look at the benefits and risks of neutering and why it is now such a common practice in small animal medicine. Additionally, before focusing on the claim that neutering a dog predisposes it to orthopaedic conditions, this thesis shall first establish the groundwork of the neutering procedure. This task entails understanding how sex hormones work, where they are produced, and their role in orthopaedics. A clear differentiation will also be made between male and female sex hormones. Once these topics have been established and understood, this paper will delve into the relationship between neutering, obesity, and orthopaedic conditions. Finally, it will focus on specific orthopaedic diseases, primarily hip dysplasia and cranial cruciate ligament disease, as well as diseases where the correlation is less established.

OBJECTIVES

The objective of this systematic literature review is to analyse the correlation that exists between neuter status and the clinical manifestation of orthopaedic disorders in dogs and to examine if there is a clear correlation between these conditions based on the age of neutering, sex and breed.

Firstly, I will outline the reasons why neutering is performed and the risk and benefits of this procedure in male and female dogs. Secondly, to understand the adverse effects of neutering, a clear understanding of the role of the sex hormones and how removing the sexual reproductive organs may adversely impact orthopaedic conditions will be considered.

I will then select six specific orthopaedic conditions and review the literature to establish if there is a clear correlation between the neutering of the dog and the clinical manifestation of these orthopaedic disorders. Specifically, I will focus on the age of neutering, the sex of the dog and the breed.

Finally, I will critically appraise my findings and evaluate the strength of the evidence. Additionally, I will consider other factors that may impact the reliability of the studies and conclusions and discuss whether these additional factors were considered in the studies selected for analysis.

1. BENEFITS AND RISKS OF NEUTERING

Neutering your pet is unique in so far as it is an elective procedure. It aims to remove the sperm and the ova source, thus making the animal incapable of producing offspring (Hart &Hart, 2021). However, as with any surgery, there are long term health risks and benefits associated with the practice. Although the main aim is to remove the organs that produce the reproductive materials, the primary source of the sex hormone's testosterone and estrogen is also removed (Hart & Hart, 2021). A review of veterinary medical literature illustrates how complex the situation is and how much research has been done to fully comprehend the adverse effects and complications of neutering.

1.1 BENEFITS OF NEUTERING

Population control

Indisputably the principal advantage of neutering is its role in population control. In 1970 in the USA, approximately 23.4 million unwanted dogs and cats were euthanised at animal shelters. This extreme amount of euthanisations carried out highlighted the necessity of neutering. This figure strongly contrasts with the decreased figure of 4.5 million dogs and cats euthanised in 2020. The key trigger for this decrease was the widespread adaptation of neutering (McKenzie, 2010). Compared to the USA and Europe, the stray dog population in many less developed countries is still growing. This can be attributed to the lack of implementation of neutering programs (McKenzie, 2010).

Mammary Gland Cancer

Mammary gland tumours are the most frequently diagnosed neoplasms in intact female dogs, and of these, 50% are malignant. A study that looked at the incidence of canine mammary tumours found that of the bitches diagnosed with this neoplasm, approximately 0.05% were spayed before their first estrus cycle. This figure increased to 8% after their first heat and 26% when the animals were spayed after their second heat.

Furthermore, the bitches who had had two or more estrus cycles but were spayed before two and a half years of age were significantly less likely to develop mammary cancer than those neutered after two and a half years of age (Schneider *et al*.,1969).

Pyometra

Pyometra is a disease that occurs relatively frequently in intact female dogs. It has been documented that pyometra develops in 25-66% of unspayed dogs older than 9-10 years old. It has also been documented that some breeds are predisposed to this condition, namely Labradors, Golden Retrievers, Rottweilers, Alsatians, and Collies (Howe, 2015). The verbatim definition of pyometra is "pus-filled uterus". It develops under the effect of progesterone in the luteal phase of the estrus cycle. It generally occurs 2-4 months after estrus and results in an acute or chronic suppurative infection. The bacteria usually detected in the inflammatory exudate in the lumen of the uterus is *Escherichia coli* (Hagman, 2018).

If left untreated, pyometra is lethal. The treatment is usually surgical in the form of ovariohysterectomy; however, there is still a 3-4% mortality rate even after treatment. Common post-surgical conditions include peritonitis, disseminated bacterial infection, sepsis, septic shock and haemorrhage, which can be deadly (Jitpean *et al*., 2014). In female dogs, pyometra can be avoided entirely by performing a complete gonadectomy in which both the ovaries and uterus are removed (Howe, 2015).

Benefits in male dogs

While the impact of neutering in population control is a benefit in both sexes, the role of neutering in preventing life-threatening diseases is much more pronounced in female dogs. It is unrefutable that spaying prevents the development of mammary gland neoplasms and pyometra in bitches. In comparison, it plays a much smaller role in the prevention of similar diseases in male dogs (Urfer & Kaeberlein, 2019).

Benign prostatic hyperplasia and hypertrophy is a disease that affects all intact male dogs over the age of six. Interestingly however, the dogs most at risk of developing severe prostate diseases, predominantly prostate cancer, are the male dogs who have undergone gonadectomy (Christensen, 2018).

In intact male dogs, testicular cancer is responsible for 16-27% of total cancers, and it accounts for 90% of tumours detected in the male reproductive tract. Neutering can prevent cancer development and generally offers a surgical cure as metastasis to other organs is rare (Houlihan, 2017).

1.2 THE RISKS OF NEUTERING

Surgical Risk of Neutering

Neutering, like any other surgery, has a level of risk involved. Although the risk of serious complications is relatively low, it does still exist. The most common complications include reaction to anaesthesia, haemorrhage, and post-surgical infection. The incidence of post-operative, intra- operative and total complications in female dogs was tracked in one veterinary hospital. The results were intra-operative complications: 6.3%, post-operative complications 14.1%, and other complications 20.6% (Sanborn, 2007).

A study carried out by five private practices in Canada studied the prevalence of post-surgical neutering complications over a 5- 13 month time period, from February 1993 to March 1994 (Pollari & Bonnett, 1996). Complications were defined as adverse health outcomes associated with an elective surgery that occurred within three weeks of the procedure (Pollari & Bonnett, 1996). 1% to 24% of all surgeries resulted in complications. Moreover, 1% to 4% of them resulted in severe complications (Pollari & Bonnett, 1996). Significant complications involving the surgical site were identified as the development of infection or abscesses, chewed out stitches or opening of the surgical wound. 90% of these complications occurred after ovariohysterectomy, as opposed to 10% after castration (Pollari & Bonnett, 1996). See Table 1 below.

Table 1: Percentage of surgeries with recorded postoperative complications in five private veterinary practices following elective procedures (Pollari & Bonnett, 1996)**.**

Surgery	Practice Number A	B	C	D	E
Dogs					
OHE[a]	35% 61/176	4% 2/54	4% 2/53	14% 7/51	30% 5/17
Castrate	32% 41/127	2% 1/58	0% 0/44	0% 0/46	15% 3/20
Total	34% 102/303	3% 3/112	2% 2/97	7% 7/97	22% 8/37
Cats					
OHE	31% 24/76	0% 0/34	0% 0/35	11% 2/19	0% 3/6
OHE & Onychectomy	28% 29/105	0% 0/35	22% 14/65	38% 13/34	50% 3/6
Castrate	1% 1/82	0% 0/49	0% 0/39	0% 0/42	5% 1/19
Castrate & Onychectomy	8% 7/83	2% 1/41	9% 4/45	10% 3/31	20% 1/2
Onychectomy	3% 1/29	0% 0/18	31% 5/16	12% 2/16	40% 2/5
Total	17% 62/372	1% 1/177	12% 23/200	14% 20/142	17% 7/41
All					
Total	24% 164/678	1% 4/289	8% 25/297	11% 27/239	19% 15/78

[a]Ovariohysterectomy

Urinary incontinence

A condition commonly linked to gonadectomy in female dogs is the development of urinary incontinence, which can be defined as "the involuntary leakage of urine during the storage phase of micturition" (Pegram *et al*., 2019). The condition has been reported to develop in dogs on average from 2.3 to 5 years after neutering. It is a relatively common disorder with an incidence rate of 3.4% to 20.1% in the US (Pegram *et al*., 2019). A study was conducted by Ponglowhapan, Church and Khalid of which the aim was to determine if there was a correlation between the mRNA and protein expression of the receptors for luteinising hormone and follicle-stimulating hormone in the lower urinary tract and the increase of the plasma concentrations of these hormones in neutered dogs. To examine this hypothesis, twenty healthy dogs that had no history or clinical signs of lower urinary tract disease were included in the study (2008). The results revealed that in neutered dogs, the expression of FSHR and LHR was noticeably reduced. This reduction was more significant in female than male dogs (2008). See Figure 1.

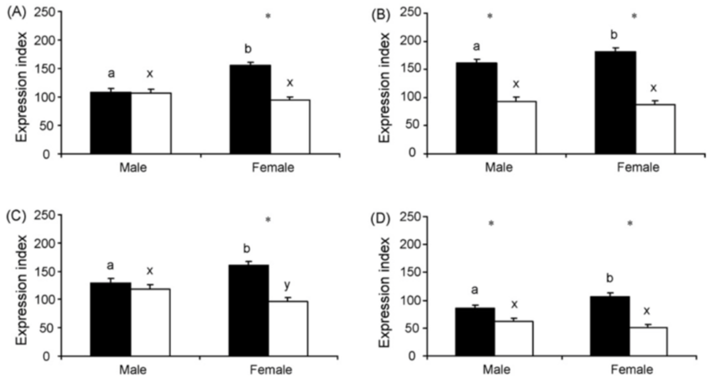

Figure 1: Mean LHR mRNA (A), LHR protein (B), FSHR mRNA (C) and FSHR protein (D) expression in the lower urinary tract of dogs. Gonadectomised dogs are represented by white and intact dogs are represented by black. Significant differences between intact and gonadectomised animals in either males or females are indicated with the asterix. The difference between genders in intact dogs is denoted by the letters "a" and "b". and "x and y" in gonadectomised dogs. (Ponglowhapan *et al.,* 2008)**.**

Neoplasia

Neutering, as discussed earlier, plays a vital role in reducing the risk of the development of certain neoplasia's in dogs. However, it can also predispose dogs to other neoplasias such as hemangiosarcoma, lymphoma and osteosarcoma (Robinson *et al*., 2020).

Hemangiosarcoma (HSA) is a relatively common cancer in dogs. It occurs in the skin, subcutis, liver and the right atrium of the heart and is responsible for 45- 51% of cancers of the spleen. Neutered bitches are five times more likely to develop HSA in the heart and two times more likely to develop HSA in the spleen than intact bitches (Robinson *et al*., 2020). A study was carried between 1964-2003. Of the 2,106,324 dogs surveyed, 5,736 developed HSA (Robinson *et al*., 2020). This figure was further broken down to show that of the female dogs diagnosed with HSA who participated in the study, 84% were spayed, and 16% were intact. 3,120 male dogs with HSA participated in the study and of them 43% of were neutered, and 57% were intact (Robinson *et al.*, 2020).

The most commonly diagnosed haemopoietic cancer in dogs is lymphoma (Zandvliet, 2016). It has been proven that there is a correlation between neutering and the development of this neoplasia. In a study carried out on 759 male and female Golden Retrievers, it was shown that this neoplasia occurs less frequently in intact dogs (Torres de la Riva *et al.*, 2013). The study showed that intact male dogs were three times less likely to be diagnosed with lymphosarcoma than male dogs who were neutered early in life (Torres de la Riva *et al.*, 2013).

Neutering has also been shown to predispose dogs to osteosarcomas and a range of other orthopaedic disease conditions, which this paper will discuss.

2.1 HOW HORMONES WORK, WHERE THEY ARE PRODUCED AND THEIR ROLE IN ORTHOPAEDICS

Hormones

Hormones can be defined as intracellular messengers. Their role in the body is to facilitate communication between cells. In the past, for a molecule to be identified as a hormone, it had to be produced in a dedicated multicellular organ and transported by the bloodstream to distant organs on which it acted. In the last two decades, studies have shown that hormones whose origin, destination path and role in the body were known and documented also had ancillary functions. They have been discovered to have unrelated roles in other non-target tissues and are produced in other non-conventional tissues (Chrousos, 2007). Hormones play crucial roles in the development, maturation and, through homeostasis, the animal's health (Borgert et al., 2013).

Sex Hormones

Through both genomic and non-genomic receptors, gonadocorticoids act throughout the entire brain of both male and female animals (McEwen & Milner, 2017). The stimulation for the production and release of the sex hormones follows a distinct pattern in adult animals. The adenohypophysis secretes luteinising hormone (LH) after stimulation from gonadotropin-releasing hormone (GnRH), which is produced by the hypothalamus (Kutzler, 2020). Once released, LH then stimulates the production and release of the gonadal steroid hormones. These are testosterone in males and progesterone and estrogen in females. Negative feedback to the hypothalamus occurs as a result of the release of the gonadal steroid hormones. This causes decreased secretion of GnRH, which causes negative feedback to the anterior pituitary gland and decreased secretion of LH (Kutzler, 2020).

Neutering animals inactivates this physiological process. As there is no secretion of these sex steroids, there is no negative feedback. Subsequently, the circulating LH level dramatically increases. In adult neutered dogs, the LH level is 30 times higher than in intact dogs (Kutzler, 2020).

A study was carried out on 28 healthy dogs to demonstrate the effect of GnRH administration before and after gonadectomy. The study aimed to establish a deeper understanding of the pituitary-gonadal axis in intact and neutered dogs and to calculate reference values (de Gier *et al*., 2012). Fourteen female dogs who had undergone at least one estrus cycle were used in the study. At the time of the study, the female dogs were anoestrus (de Gier *et al*., 2012). Fourteen male dogs with two scrotal testicles comprised the male population of the study. The week before neutering a GnRH stimulation test was carried out on all dogs. A second GRH stimulation test was carried out 133 days after gonadectomy in female dogs and 148 days following gonadectomy in male dogs. The GnRH analogue administered to female dogs was gonadorelin, and buserelin was administered to male dogs (de Gier *et al*., 2012).

The results of the study were as follows. Following gonadectomy in both male and female dogs, the basal LH and FSH concentrations were markedly increased. In female dogs prior to gonadectomy, the basal plasma LH concentrations were lower, and basal plasma FSH concentrations were higher than male dogs. However, following gonadectomy, this difference in concentrations between the sexes was not pronounced. Plasma LH and FSH concentrations were elevated following GnRH administration prior to gonadectomy in both sexes. GnRH administration following gonadectomy resulted in an increased concentration of plasma LH in both sexes, while only in neutered male dogs a significant increase in plasma FSH concentration was seen. A considerable increase in plasma testosterone concentration following GnRH administration prior to gonadectomy was seen in both sexes. Regarding basal plasma estradiol concentrations, it was seen that the levels were significantly elevated in intact vs castrated male dogs. In female dogs, no significant difference in concentration was seen before and after gonadectomy (de Gier *et al*., 2012).

The study concluded that for establishing neuter status in both sexes of dogs, basal plasma FSH concentration is more dependable than basal plasma LH concentration. Additionally, for verifying neuter status between the separate sexes, the measurement of basal plasma testosterone is accurate in male dogs. To differentiate between female dogs with functional ovarian tissue and without functional ovarian tissue, determination of plasma estradiol concentration 120 minutes following administration of GnRH can be used (de Gier *et al.,* 2012).

2.1 THE FEMALE HORMONES

The role of estrogen in tendons and ligaments

Tendons and ligaments are two vital components of the musculoskeletal system. The metabolism of the connective tissues such as bone, muscles and cartilage are known to be regulated by the hormone estrogen. Estrogen's principal purpose is to develop and maintain the normal functioning of the female reproductive organs. Additionally, developmental processes, for example, the formation of bone, are under the influence of estrogen. The ovaries and placenta are the two primary sources of this sex steroid. Through intracrine synthesis, the adrenal glands, the male testes, and several peripheral cells and tissues also produce small amounts of the hormone (Leblanc *et al*., 2017).

In 1996, Liu S.H *et al*. demonstrated, using tendon tissues, the expression of the estrogen receptors, ER-α on the anterior cruciate ligament of humans using immunoperoxidase assay (Leblanc *et al.,* 2017.). The primary role estrogen plays in the tendon tissue is through its influence on collagen synthesis. Collagen is the main component of the extracellular matrix (ECM) of the tendons accounting for 60-85% of the dry mass. More specifically, 95% of the collagen is Type 1 Collagen. Studies have demonstrated that by adding or increasing the amount of estrogen like compounds (ELC), the synthesis of collagen is improved. These studies were carried out using pig cell-based cell cultures and engineered tendon tissue using cells of human origin. Another study using rabbit cell cultures revealed that the collagen synthesis decreased when estrogen was below the physiological level of < 0.025 ng/ml (Leblanc *et al*., 2017.).

When the cell cultures of ovariectomised, young and old animals are compared, there are significant differences seen in the proliferation of cells and the synthesis of the ECM. In the ovariectomised and older animals, factors known to aid in the healing process, such as proliferation rate and collagen type 1, are diminished compared to the younger animals. See Figure 2 below. Conversely, in ovariectomised animals, Collagen Type 3 and matrix metalloprotease-13 were markedly elevated compared to intact animals (Leblanc *et al*., 2017).

Estrogen has also been shown to affect the collagen in the cartilage. The prevalent collagen type in the articular cartilage is Type 2. It has been demonstrated that ELC prevents the breakdown of Type 2 collagen on a molecular level (Leblanc *et al*., 2017).

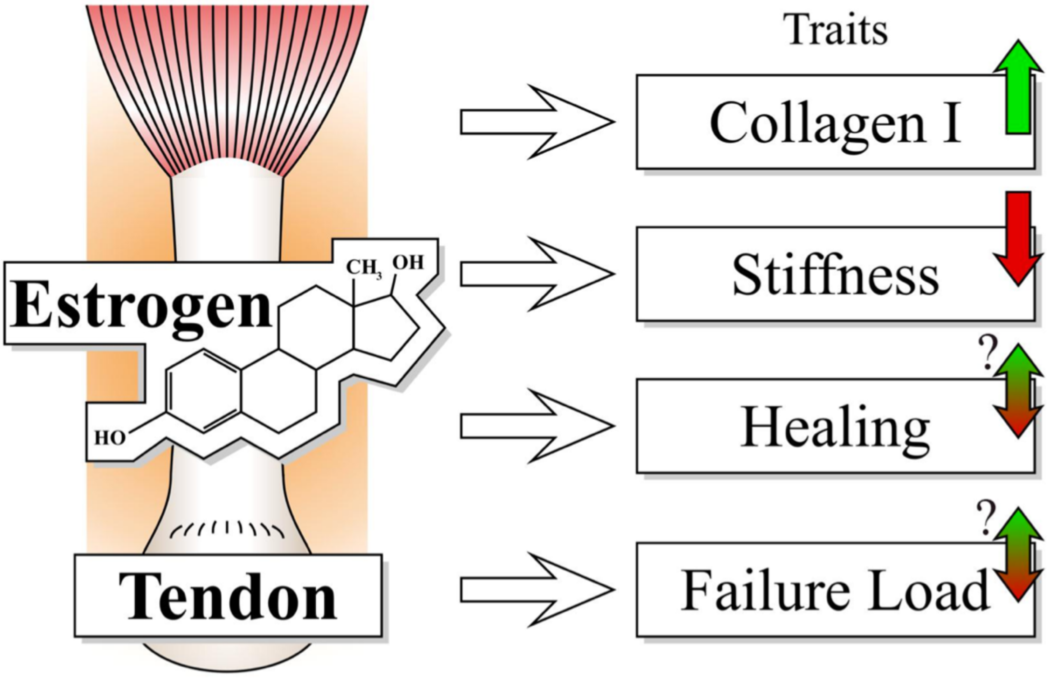

Figure 2: The effects of estrogen on tendon tissue (Leblanc *et al*., 2017.).

The role of estrogen in the skeletal muscles

Research has demonstrated that there is a strong correlation between estrogen levels and muscle mass. It has been shown that muscle maintenance, regeneration, and hypertrophy in adult animals, as well as postnatal muscle growth, are strongly influenced by muscle stem cells, known as satellite cells. Studies have shown that estrogens positively impact muscle regeneration following muscle injury. They achieve this by downregulating the inflammatory response, increasing the number of satellite cells, and stimulating the proliferation of the satellite cells after exercise (Kitajima & Ono, 2016).

In Japan, a study was carried out on young ovariectomised female mice to analyse the effect reduced estrogen levels had on the muscle. The study showed that muscle atrophy resulted from the decreased estrogen levels, and this effect occurred in a time-dependent manner (Kitajima & Ono, 2016). Muscle force generation was also reduced in the mice analysed 24 weeks after gonadectomy. This was seen using the muscle function grip test (Kitajima & Ono, 2016). Histological immunohistochemistry was used to examine the effect of estrogen insufficiency on the size of the muscle fibres. It was seen that the cross-sectional area of the anterior tibialis muscle was significantly smaller in the mice examined 8 and 24 weeks after the gonadectomy surgery compared to the intact control mice (Kitajima & Ono, 2016). Concerning the satellite cells, their expansion, differentiation, and self-renewal were significantly reduced, but the actual number of satellite cells remained the same in the isolated myofibers. In conclusion, the muscle regeneration was markedly reduced in ovariectomised female mice (Kitajima & Ono, 2016).

The results of the study ultimately revealed that estrogens play a vital role in the maintenance of healthy muscle and satellite cell function in young female mice, and decreased muscle regeneration and strength occurs as a result of inadequate levels (Kitajima & Ono, 2016).

Role of estrogen on the bone

A key regulator of bone mass is estrogen. In humans, one of the leading causes of osteoporosis is estrogen deficiency following menopause (Streicher *et al*., 2017).

The main effect of decreased estrogen levels on bone is that bone resorption is increased. However, estrogen also plays a role in regulating the formation of bone on a cellular level. When the estrogen level of the animal is decreased, a gap between the formation and resorption of bone occurs. Estrogen has direct and indirect effects on all three bone cells, the osteocytes, the osteoblasts and the osteoclasts. Suppression of the remodelling of the bone, reduced resorption of the bone and preservation of bone formation are all a result of the direct effect of estrogen (Khosla *et al*., 2012). See Figure 3.

It was previously thought that as testosterone is the primary sex hormone in males, it played an identical role in the bone metabolism in men to that of estrogen in females. However, in the mid-1990s, it was proved that estrogen is also the primary hormone involved in regulating bone metabolism in males (Khosla *et al*., 2012).

The osteocyte plays a critical role in controlling the activation of the remodelling and restoration of the bone. Tomkinson *et al*. discovered that using rats as test subjects; female ovariectomised rats had a four-fold increase in osteocyte apoptosis in the tibia in both cancellous and cortical bone (Khosla *et al*., 2012). Emerton *et al.* researched the biological significance of the correlation between estrogen deficiency and the apoptosis of the osteocytes. Ovariectomized mice were treated with a pan-caspase inhibitor, which stopped the osteocyte apoptosis seen in the cortical bones of other ovariectomised mice who were not treated. The treatment also prevented the resorption of bone on endocortical surfaces by osteoclasts which usually occurs after gonadectomy. These various studies demonstrate that a significant target cell for estrogen is the osteocyte. They also show that estrogen deficiency triggers osteocyte apoptosis, which causes bone remodelling and resorption (Khosla *et al.*, 2012).

Estrogen targets the osteoclasts directly by stimulating apoptosis. This has been proved in studies such as the ones carried out by Namamurka *et al.* and Martin-Millan *et al.* These studies showed that the trabecular bone mass decreased when estrogen receptor alpha on the osteoclasts was explicitly deleted. The bone mass increased due to the osteoclast apoptosis decreasing. The reduced apoptosis was caused by an increased lifespan of the osteoclasts (Khosla *et al.*, 2012).

Estrogen indirectly affects the osteoclasts by reducing the c- junction activity macrophage colony-stimulating factor, which blocks the receptor activator of nuclear factor-κB ligand (RANKL)/ macrophage colony-stimulating factor-induced activator protein dependent-1 transcription. This causes diminished c-junction expression and reduced phosphorylation. Additionally, estrogen causes reduced osteoclast differentiation, which occurs under the stimulation of RANKL (Khosla *et al.*, 2012).

As stated previously, estrogen deficiency results in a discrepancy between the rate of bone resorption and remodelling and bone formation. This is primarily due to reduced bone formation due to the direct effect of estrogen on osteoblasts. Estrogen deficiency affects the osteoblast in 3 ways. It causes an increase in apoptosis, an increase in oxidative stress and an increase in the activity of NF-κB (Khosla *et al.*, 2012).

It has been proven that estrogen causes an increase in the functioning of osteoblasts. It changes the activity of select transcription factors by activating the Src/Shc/ERK signalling pathway and downregulating c-Jun N-terminal kinases. These molecular actions cause decreased apoptosis of the osteoblasts and therefore increased longevity of osteoblasts (Khosla *et al.*, 2012).

Increased numbers of indicators for oxidative stress in the bone are seen in older animals compared to younger animals. This was illustrated in a study on mice carried out by Manolagas *et al*. The reactive oxygen species (ROS), which are formed due to oxidative stress, have been proven to reduce osteoblastogenesis and cause reduced life expectancy of the osteoblasts and osteocytes. In contrast, osteoclasts are positively impacted by ROS. The production, operation and viability of the osteoclast are, in fact, dependent on the ROS (Khosla *et al*., 2012). When estrogen-deficient animals are given antioxidants, these effects are reversed (Khosla *et al*., 2012).

Estrogen deficiency affects the functioning of NF-κB as seen in studies carried out by Chang *et al.* These studies, which were carried out in vivo, revealed that the activity of NF-κB was higher in the osteoblasts under estrogen-deficient conditions. They additionally demonstrated that when the NF-κB was inhibited in ovariectomised animals, there was reduced bone loss. It was also seen that increased expression of Fos-related antigen occurred when the NF-κB was inhibited. Fos- related antigen has been proven to play a vital role in bone matrix formation due to its role as a transcription factor (Khosla *et al.,* 2012)

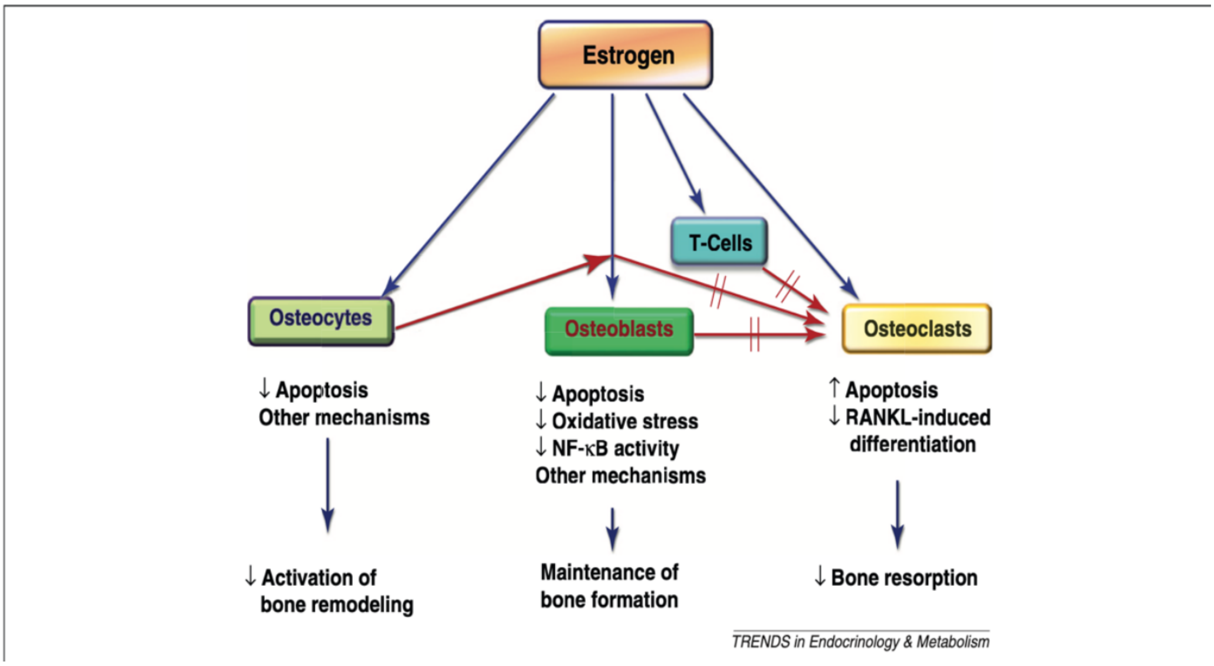

Figure 3: The role of estrogen in the regulation of bone turnover. It's specific effects on osteocytes, osteoblasts, osteoclast, and T cells (Khosla *et al*., 2012)**.**

<u>Study in dogs</u>

A study was carried out in Taiwan aiming to explore the role that gender and estrogen therapy exerts on biological fixation. Cobaltxbromium orthopaedic plugs were inserted into both sides of the distal femur and the bone ingrowth was measured and compared (Shih *et al.*, 2003).

Thirty-two dogs were chosen as test subjects that were entirely orthopedically developed and between 4- 5 years old. Twenty-four were female, and eight were male. The test subjects were divided into four groups. Group 1 comprised eight female dogs who were left intact but had undergone a laparotomy to confirm the presence of ovaries. This was the female control group. Group 2 comprised eight ovariectomised female dogs who had both ovaries removed. The dogs in this group received no estradiol treatment. Group 3 was eight ovariectomised female dogs, but these dogs received high dose estradiol treatment. The final group was comprised of eight intact male dogs who, too, underwent an exploratory laparotomy. These were the male control group. The progesterone levels were tested twice a month in the ovariectomised dogs to record the hormone concentration changes before and after gonadectomy. The two plugs were surgically placed in the distal end of the femur bone of all test subjects six months after ovariectomy. The reason for this interval was to allow enough time for the metabolism of the bone to adapt after the removal of the sex steroid producing gonads.

Three months post-implantation, the dogs were euthanised, and samples were taken. The results showed that the level of bone ingrowth in the male and female control groups was relatively equal. Both groups of ovariectomised females had notably decreased levels of ingrowth, specifically the plugs in contact with the cancellous bone. The plugs in contact with the cortical remained relatively unaffected and still had substantial bone ingrowth. Treatment with subcutaneous high dose estradiol did not have any effect (Shih *et al.*, 2003).

This study showed that it was the endogenous estrogen produced by the dog that affected bone growth. When the ovaries and the source of estrogen were removed, bone growth decreased. Treatment with exogenous estrogen did not have any effect on bone growth.

2.2 THE MALE HORMONES

Effect of Androgens on ligaments

Androgens are the principal male sexual hormones. Their primary roles include provoking male sexual differentiation before birth, inducing sexual maturation during puberty, and regulating the normal functioning of the genital system in adult males (Vanderschueren *et al.,* 2004). This includes spermatogenesis. The principal places of secretion of androgens are the testis and the adrenal glands. Androgens are C-19 steroids, and the principal androgen in males is testosterone. The enzyme 5α- reductase can transform testosterone into 5α - dihydrotestosterone (DHT) in the peripheral tissues, which is higher in potency. This transformation cannot be reversed (Vanderschueren *et al.,* 2004).

While much research has been carried out on estrogen's role on ligaments, explicitly concerning their strength and rate of injury, the same has not been done for testosterone. While it has been shown that the cranial cruciate ligament (CCL) has androgen receptors, it is still not entirely known if it is, in fact, a testosterone dependent tissue. It has, however, been proven that testosterone plays a role in protecting against cartilage degradation caused by inflammation and that it increases the expression of collagen in the intervertebral disk tissues (Romani *et al*., 2016).

Romani *et al.* conducted an experiment where the strength of the CCL was compared in castrated rats with reduced testosterone levels and intact rats with physiological testosterone levels (2016). After 35 days, the rats were euthanised, and a cross-section was taken of the CCL, and the area was calculated (2016). The ligament properties were measured using a servo-hydraulic material testing unit. (2016).

Three tests were carried out on the CCL. These were the load to failure, the ultimate stress test and the mean energy test. While both groups' cross-sectional areas and energy test results were similar, the load to failure and ultimate stress results showed a divergence between the two groups. The results were significantly higher in the intact rats than in the castrated group (2016). This showed that testosterone concentration plays an essential role in the ability of the ligament to resist increased tensile loads and may play a role in preventing CCL injury (2016).

The effect of androgens on the skeletal muscle

The exact role of androgens on the skeletal muscle of mammals has been debated for many years. Currently, it is readily accepted that the effect androgens have on muscle cells is firstly anabolic and secondly direct. They have a hypertrophic effect on Type 1 and Type 2 muscle fibres, which increases muscle mass. The most popular hypothesis to explain this effect is that by stimulating the synthesis of muscle proteins, reducing the degradation rate of the muscle proteins and promoting amino acids re-utilisation, the net muscle protein balance is improved by testosterone (Bhasin *et al*., 2003).

Bhasin *et al*. carried out a study to test the hypothesis that testosterone induces muscle hypertrophy and aids in the proportion of pluripotent stem cells that enter the myogenic lineage and decreases the proportion that are transformed to adipose cells.

To verify the validity of the hypothesis that muscle fibre hypertrophy induced by testosterone results in an increase in muscle size, a group of young men in perfect health were given a long-acting GnRH agonist injection monthly. This GnRH agonist resulted in suppressed endogenous secretion of testosterone. The men also received weekly injections of testosterone for 20 weeks. Different dosages ranging from high to low were given to different test subjects throughout the entirety of the experiment. A muscle biopsy was taken from the vastus lateralis muscle of each man both before and after the experiment. Magnetic Resonance Imaging was used to determine the muscle volume (2003). It was shown that the muscle volume increased over the 20 weeks, and the increase was directly proportional to the dose administered and the concentration of testosterone. Cross-sectional analysis of the muscle fibres showed an increase of Type 1 and Type 2 fibres. The proportions of the groups of fibres remained unaltered. This experiment proved that increased muscle volume mediated by testosterone is concentration-dependent (2003).

It has been proven that testosterone also has a strong influence on satellite cells and myonuclei. This was illustrated in the experiment conducted by Bhasin *et al.* In the biopsies of the vastus lateralis taken before and after the experiment, the myonuclei were counted. An increase in the myonuclear number was seen in the samples taken after 20 weeks. As it is known that satellite cells contribute to myonuclei in the muscle fibres, Bhasin *et al*., by direct counting and spatial orientation methods, quantified the number of satellite cells. It was shown that elevated testosterone concentrations, both total and free, increased the numbers of satellite cells (2003).

It has been proven that in all skeletal muscles, androgen receptors are expressed, and that expression level varies within the different muscle groups. A topic debated is what effect androgens exert on the muscles, and if it is amplified by an androgen receptor-mediated pathway, specifically through the anti-glucocorticoid effect. The anabolic effects of testosterone have been shown to be antagonised by glucocorticoids in animal models. In animal models, it is also seen that muscle- atrophy induced by glucocorticoids can be prevented by administering testosterone. In contrast, it has also been shown that the androgen receptor expression is upregulated in the skeletal muscle following the administration of androgens. This poses the question, is the sensitivity of the skeletal muscle to the action of androgens elevated following administration of testosterone by upregulation of its own receptor? (2003).

It remains unknown whether the androgens affect the skeletal muscles in the form of DHT or testosterone. All that is proven is that low concentrations of the enzyme 5α- reductase are found within the muscle (2003). It also remains unclear if the aromatisation of androgens to estrogen is vital for androgens to carry out their role in the muscle. A study on mice showed that mice who carried the P450 aromatase enzyme complex gene had a lower fat mass and higher muscle mass than those who did not carry the gene (2003).

The Role of Androgens in the bone

Both estrogen receptors and androgen receptors are expressed on the bone cells. The estrogen receptors can be subdivided into ERα and ER β (Sinnesael *et al.,* 2011). DHT and testosterone activate the androgen receptors and testosterone, via the P450 aromatase enzyme complex, can be converted to estradiol and also activate the estrogen receptors (Hammes & Levin, 2019).

Both androgens and estrogens maintain the mass and integrity of cancellous bone. Androgens, by stimulating the longitudinal and radial growth of the bones, increase the gross cortical bone size. This occurs by the biphasic effect that androgens exert on the formation of the endochondral bone. This effect is also seen with estrogens. At the onset of puberty, sex steroids induce the formation of the endochondral bone, and at the end of puberty, they stimulate closure of the epiphyseal plates. However, this effect on the physis occurs via the effect of the androgens on ERα after transformation to estrogens. Androgens play a critical role in bone strength, mediated via their role in amplifying the radial growth of bones. In addition to this, the increased bone strength can be linked to increased periosteal bone formation, whereas in females, estrogen limits the periosteal formation of bone (Vanderschueren *et al.,* 2004).

Androgens affect several specific bone cells both directly and indirectly. These include the growth plate chondrocytes, the osteocytes, the osteoblasts, and the osteoclasts (Vanderschueren *et al.,* 2004).

Within the growth plates, chondrogenesis occurs following the proliferation and hypertrophy of the chondrocytes and the cartilage matrix secretion. After the cartilage formation, endochondral ossification takes place. This results in the longitudinal growth of the bones. The endocrine system regulates this process and androgens have been proven to play a critical role (Nilsson *et al*., 2005). A study was carried out on rats to examine the effects of testosterone on the chondrocytes in vitro. Its aim was to ascertain if the effect of testosterone was influenced by gender and if the stage of maturation of the chondrocytes was significant. In the study, the cell culture model was formed from chondrocytes from the growth and resting zone cartilage from male or female rats. Foetal bovine serum was used as the culture medium. The results of the study showed that testosterone did increase collagen production in both cartilage zones; however, this was only seen in male rats. Testosterone did not affect collagen production in female rats. This study showed that testosterone, through its effect on collagen production in the growth plates, increases the longitudinal growth of bones in males (Schwartz *et al.,* 1994).

Androgens have multiple direct and indirect effects on osteocytes and osteoblasts. Androgen receptors have been demonstrated in the primary cultures of human osteoblastic cells. Androgens directly affect the proliferation and differentiation of osteoblasts (Nakano *et al.,* 1994). Whether this direct effect occurs in the form of testosterone or DHT is debatable. A study carried out by Nakano *et al.* found that in relation to the cell proliferation, DHT and testosterone had similar potency and suggested that testosterone mainly acts on the osteoblasts without conversion to DHT (1994). A study that demonstrated the indirect effect of androgens was carried out by Kousteni *et al.* on mice and showed that in both sexes, gonadectomy resulted in a decreased lifespan of osteoblast and osteocytes due to an increased rate of apoptosis. This creates an imbalance between the resorption and growth of the bone, which results in a weakened skeleton (2001). The local effects of androgens on the bone are mediated by three locally acting cytokines and growth factors; transforming growth factor- β (TGFb), insulin like growth factors (IGFs), and interleukin 6 (IL-6), which are regulated by androgens. From a functional point of view, androgens conserve bone mass by inhibiting the action of IL-6, which increases osteoclastogenesis and the resorption of bone. Androgens stimulate TGF**b** and IGFs, which play a crucial role in the formation of bone by regulating the proliferation and differentiation of the osteoblasts (Vanderschueren *et al.,* 2004). A study carried out by Kasperk *et al.* using murine calvarial cell cultures treated with androgens examined the concentrations of insulin growth factor 1 and 11 (IGF-1 and IGF-11) and TGF- β. The aim was to examine if these growth factors mediated the effect of androgen on the bone and if androgens alter the response to growth factors. An increase in the concentration of TGF- β was seen. Additionally, it was seen that the mitogenic effects of IGF-11 were increased following treatment with DHT. The study concluded that androgens positively affect the osteoblasts by inducing the secretion of TGF- β and sensitizing the cells, which results in an increased response to IGF-11 (1990).

Androgens directly impact the osteoclast, and this is visibly seen after castration. Osteoclasts are formed from cells within the bone marrow. These cells are the hematopoietic precursor cells of colony-forming unit granulocyte-macrophage descent (Huber *et al*., 2001). Following castration, the osteoclast precursor number is upregulated (Huber *et al*., 2001). For the bone-sparing effect of androgens to be successful and for the osteoclasts to reach their final maturation stage, contact with osteoblastic stromal cells of the bone marrow is essential (Suda *et al*., 1999). Independently of the effects on the bone marrow cells, androgens have been proven to regulate the survival of the osteoclast, regulate the expression of RANK in preosteoclasts and modulate the activity of mature osteoclasts (Huber *et al.,* 2001).

The results of these studies show that androgens play a significant role in the regulation of the metabolism of the bone, through their effects on all four types of cells.

3. SPECIFIC ORTHOPAEDIC CONDITIONS

This paper will now focus on specific orthopaedic conditions that have been proven to be influenced by the gonadectomised status of the dog. These conditions include cranial cruciate ligament rupture, hip dysplasia, elbow joint dysplasia, patellar luxation and intravertebral disc disease and osteosarcoma,. A key distinction must be made between early and late neutering as the timing of gonadectomy plays a role in the orthopaedic effect. In the USA, early-age gonadectomy, where a dog is neutered or spayed between 6-9 months of age, remains a common practice. Additionally, it is recommended that spaying occurs before the first estrus of the animal (Root Kustrit, 2007).

In studies carried out on German Shepherd, Labrador Retriever and Golden Retriever dogs, it has been proven that dogs neutered before 12 months of age have 2-4 times the probability of developing orthopaedic conditions compared to intact dogs of the same age. Conversely, the neutering status did not appear to affect the development of orthopaedic conditions in small breed dogs (Hart *et al*., 2020).

It has been proven that sex hormones play a role in controlling the elongation of the bones. Thus, gonadectomy and removing the sex hormones before the closure of the growth plates influences the physeal growth. When early gonadectomy is performed, it results in increased elongation of the long bones. This has been shown to result in increased orthopaedic conditions and injuries. The earlier gonadectomy is performed, the more prominent this effect (Root *et al*., 1995).

3.1 Cranial Cruciate Ligament Rupture

One of the most frequently diagnosed joint conditions in dogs is the rupture of the cranial cruciate ligament (CCL) in the stifle (Witsberger *et al*., 2008). Disorders of the CCL can negatively influence the life of the animal. This is due to the crucial role that this ligament plays in the femoro-tibial patellar joint. It influences both the stability and the biomechanical functioning of the joint and hence can significantly impact the ability of the animal to exercise (Spinella *et al*., 2021). This is the role that the ligament plays in the stifle joint. The joint itself then plays a vital role in the pelvic limb. It absorbs and supports the body weight when the animal is standing. During movement, the joint is responsible for allowing the transmission of the propulsive thrust towards the coxo-femoral joint and the shortening of the functional length of the pelvic limb (Spinella *et al.*, 2021). CCLR can occur in two forms, traumatic CCLR or the more common rupture of the CCL following a prolonged period of degeneration. This degeneration occurs due to the effect of several predisposing factors, which will be outlined below (Comerford *et al*., 2011).

A study was carried out by Witsberger *et al.* where the medical records of 1,243,681 dogs in the Veterinary Medical Database between 1964-2003 were analysed to evaluate the prevalence of specific orthopaedic conditions. The Veterinary Medical Database comprises patient records submitted by 27 different Veterinary Teaching Hospitals in North America (2008). It was seen that out of the 1,243,681 dogs evaluated, CCLR was diagnosed in 31,698. Thus, CCLR had a prevalence of 2.55% in the population. The results from the study also showed that CCLR is much more prevalent in neutered dogs and bitches than their intact counterparts (2008). See Table 2.

The sex and neutering status of the dog significantly impact the development of this condition; it has been proven in many studies that females are most at risk due to the different levels of estrogen produced at different stages of the estrous cycle. The causes behind this are still under investigation, and the research is mainly focused on human medicine. Currently, there are two hypotheses; one is that CCLR occurs mainly during the follicular phase. The second is that CCLR occurs predominantly during the ovulatory phase as high estrogen levels have been proven to cause decreased collagen production and thus influence the strength of the ligament (Spinella *et al.,* 2021).

Studies have additionally shown that gonadectomised male and female dogs are unrefutably more at risk of developing this orthopaedic condition (Spinella *et al*., 2021).

The constant breaking down and synthesis of protein and the steroid hormones regulate the metabolism and the remodelling of the connective tissue. This includes the connective tissue in the ligaments. Gonadectomy and the removal of these regulatory steroid hormones have been shown to result in an increased risk of ligament rupture. On a molecular level, the LH receptors of the ligaments found in both neutered and intact animals are overactivated in neutered animals due to the increase of circulating LH. As discussed earlier, the circulating LH increases as negative feedback on its production is caused by the steroid hormones, which are no longer present in neutered animals. The overactivation of the LH receptors results in increased laxity of the CCL, thus increasing the risk of injury. This effect occurs in the early and late neutered dogs (Spinella *et al.,* 2021).

Early neutering alone creates another predisposing factor for this condition. This is due to the delayed closure of the physeal plate of the bone, in this case, the tibia. This results in the tibial plateau caudo- distal inclination being increased, and as a result, the cranial tibial thrust is increased. This anatomical change can result in CCLR (Spinella *et al.,* 2021).

A study was carried out by Torres de la Riva *et al.* exclusively on Golden Retrievers. The Veterinary Hospital Records of 759 dogs, both intact and neutered, male and female, were analysed to assess the prevalence of joint disorders and neoplastic diseases in neutered and intact dogs. One specific breed was chosen to analyse so the study could focus on just the age and neutering status associated with this disease, independent of the genetic differences of different breeds. The Golden Retriever was chosen due to its proven susceptibility to orthopaedic and neoplastic conditions (2013). When the occurrence of CCLR was analysed, it was seen that CCLR did not occur in both sexes of intact dogs and late-neutered females. Dogs were considered to be "late-neutered" if the gonadectomy occurred after 12 months of age. Within the two groups of male and female early-neutered dogs, the prevalence of CCLR was 5.1% and 7.7%, respectively. The mean age of the emergence of the condition in early neutered male dogs was 3.6 years of age, and in early neutered female dogs was 4.8 years (2013).

Similar results were identified in a second study carried out by Hart *et al.* on 1170 German Shepherds. Of the male dogs analysed, it was apparent that neutering significantly increased the occurrence of CCLR. Less than 1 % of the intact male dogs were diagnosed with CCLR. Upon comparison of the neutered male dogs, the group neutered before 6 months of age had a 12.5% occurrence of this condition, and the group neutered between 6-11 months had an

8.3% occurrence of CCLR (2016). A similar trend was identified among the female dogs. As with the male dogs, the prevalence of CCLR was less than 1% in intact bitches. In the female dogs spayed before 6 months of age, the occurrence of CCLR was 4.6% and 8.3% in the bitches spayed between 6-11 months of age (2016).

Treatment of CCLR can either be surgical or conservative. Surgery is currently recommended for optimum results and can restore the joint (Spinella *et al*., 2021). However, conservative management is still an option and may be chosen based on a range of logistical factors such as pre-existing conditions, the age of the dog, and the financial cost of surgery (Spinella *et al*., 2021).

From my analysis of the literature cited above, it is evident that of the two sexes, female dogs are more susceptible to CCLR than male dogs due to the role of estrogen. Subsequently, it is apparent that neutering both male and female dogs increases this condition's prevalence through the effect of the sex steroids on the LH receptors. The age at neutering is significant, and early neutering is associated with an increased risk of CCLR due to inappropriate timing of the closure of the physeal plates. Also, from my analysis of the literature, I noted other causative agents that predisposed dogs to this condition.

These include the breed and bodyweight of the dog. CCLR occurs more frequently in larger and overweight dogs, the joints are under more pressure, and the impact on the cranial cruciate ligament is greater, leading to degeneration. The dog breeds in which this condition is most commonly seen are the Labrador, Newfoundland, Rottweiler, Neapolitan Mastiff, Saint Bernard, Chesapeake Bay Retriever, American Staffordshire Terrier, Akita, Boxer, and Bulldog (Spinella *et al.,* 2021). However, in some of these breeds, including the Saint Bernard, Boxer, Rottweiler and Chow Chow another factor may influence the development of CCLR. This is the developmental fault of very open joint angles, which results in the pelvic limbs being in a hyperextended position, thus putting increased pressure on the CCL (Spinella *et al.,* 2021).

Obesity, as with most orthopaedic conditions, is another factor that most likely will lead to CCLR. This is through the release of inflammatory mediators from the white adipose tissue (Comerford *et al.,* 2011). The proven release of adiponectin, leptin and resistin from the intra-articular adipose tissue and other tissues within the joint, as well as from the systemic adipose tissue, has been hypothesised to play a role in CCLR through the development of chronic inflammation in the individual (Comerford *et al*., 2011).

It is also evident that age is another apparent predisposing factor in the development of this orthopaedic condition. Research has proven that the age range in which CCLR most commonly occurs in dogs is between 2-10 years of age. More specifically, it is between 5-7 years of age that the ligament ruptures following a period of degradation (Spinella *et al.,* 2021).

Table 2: The prevalence of HD and CCLD in 1,243,681 dogs that had been examined between 1964-2003 at veterinary medical teaching hospitals in North America. The dogs were grouped according to their sex and neuter status, (Witsberger *et al.,* 2008).

		Dogs with HD		Dogs with CCLD	
Group	Total No. of dogs	No. (%)	OR (95% CI)	No. (%)	OR (95% CI)
Sexually intact female	307,957	10,324 (3.35)	0.93 (0.91–0.96)	4,614 (1.55)	0.51 (0.49–0.53)
Spayed female	319,725	11,010 (3.44)	0.97 (0.95–0.99)	14,004 (4.54)	2.35 (2.30–2.40)
Sexually intact male	458,525	15,984 (3.49)	0.98 (0.96–1.00)	6,948 (1.57)	0.47 (0.46–0.49)
Castrated male	157,474	6,506 (4.13)	1.21 (1.18–1.24)	6,132 (4.06)	1.68 (1.63–1.73)

The OR represent the odds of HD or CCLD in that group, compared with the odds in all other dogs not in that group. Odds ratios for which the 95% CI does not contain 1 are significantly ($P < 0.05$) different from 1.

3.2 Hip Dysplasia

In large breed dogs, canine hip dysplasia (HD) is the most frequently diagnosed orthopaedic condition. In some specific breeds, it can even have as significant a prevalence as 70% (Smith *et al*., 2001). HD in dogs occurs when there is an imbalance in the growth of the skeleton and the primary muscle mass. Although the dogs are born with anatomically correct hips, skeletal growth occurs at too rapid a rate. The dysplasia results from compensatory changes to the bone as it struggles to maintain the correct positioning of the femoral head in the acetabulum. The surrounding muscles are not strong enough to sustain the correct articulation. HD can range from simple changes in the structure of the bone to the complete demolition of the functioning joint (Alexander, 1992). A dog is classified as suffering from HD radiographically if periarticular osteophytosis, sclerosis of the subchondral bone and remodelling of the joint is seen in the ventrodorsal hip- extended radiographic view (Smith *et al.,* 2001).

HD most commonly presents as a degenerative joint disease (DJD). Even though this disease is a genetic condition, many environmental factors can impact its phenotypic manifestation. These factors include diet, exercise and neutering status, and any other conformational characteristics exhibited by the dog. In some dogs, evidence of the DJD is not seen radiographically until later life, and other animals may die before the onset of the DJD (Smith *et al.*, 2001).

The risk of HD developing in dogs can be predicted using the Distraction Index, which measures the degree of laxity of the passive hip joint. This measurement is calculated by taking a radiographic stress x-ray of the pelvis and measuring the relative displacement of each femoral head from its usual position in the acetabula (Smith *et al.,* 2001).

It has been proven that gonadectomy is a contributing factor in the development of this condition in predisposed dogs. The probability of the development of HD during the lifetime of dogs is 1.5- 2 times higher in neutered than intact dogs (Kutzler, 2020). Once again, a reason for this is thought to be due to the occurrence of increased levels of circulating LH in gonadectomised dogs. Receptors for LH are present in the tissues that provide structural support to the hip joint, specifically in the ligamentum teres femoris, the hyaline cartilage, and the subchondral bone of the femoral head. The overactivation of these LH receptors causes laxity of these supporting tissues, leading to the development of HD (Kutzler, 2020).

A study was carried out on boxers in the Netherlands in which 1,863 purebred boxers born between January 1994 and March 1995 were evaluated every six months by written questionnaire until they reached 7-8 years of age (van Hagen *et al.,* 2005). The aim of the study was to determine the incidence, risk factors, and heritability estimates of hind limb lameness caused by hip dysplasia in a birth cohort of Boxers (von Hagen *et al.,* 2005). Female dogs comprised 53.3 %, and males comprised 46.7% of the population (van Hagen *et al.,* 2005). Upon evaluation of the results, that in comparison to intact dogs, neutered dogs were 1.5 times more likely to develop this condition (van Hagen *et al.,* 2005).

HD is a condition that is much more prevalent in early-neutered male dogs. In the study carried out in 2013 by Torres de la Riva *et al.* on Golden Retrievers (2013). It was seen that HD affected 10.3 % of early neutered male dogs in this study and only 5.1% of intact male dogs. The timing of the neutering of the male dog, early or late, also significantly impacted the disorder's development. See Figure 4. In female dogs, the timing of spaying did not play a part. See Figure 5. When analysed by age, it was seen that the mean age for onset of the disorder in males was in intact dogs 4.4 years old, in early-neutered dogs 3.6 years old and late neutered dogs 4.7 years (2013).

The regulatory effect of the sex hormones on the closure of the physeal plates significantly impacts the development of this disorder. It has been proven that the closure of the growth plates occurs quicker in males than females. In the study carried out in 2013 by Torres de la Riva *et al.,* it was shown that early gonadectomy resulted in double the occurrence of HD in neutered males, whereas, in females, the occurrence of HD was not influenced by ovariohysterectomy (2013).

From my analysis of the literature on this topic, it is evident that neutering does significantly increase the development of this condition in neutered male dogs, specifically those male dogs neutered before 12 months of age. This appears to be due to the more significant impact that early neutering has on the closure of the growth plates in male dogs compared to female dogs. The occurrence of the condition in female dogs does not appear to have any correlation with ovariohysterectomy.

However, as with all specific orthopaedic conditions, other predisposing factors which play a role in the occurrence of HD that must be considered. One such factor is obesity which has a proven link with the occurrence of the condition. Therefore, in the Torres de la Riva *et al.,* study, the dogs predisposed to the condition due to increased body weight from neutering and those gonadectomised dogs with a good body condition score (BCS) who developed the condition were separated. The group of early neutered dogs with HD and the early-neutered group without the condition were compared to evaluate the difference in BCS (2013). The results showed that the BCS of both groups was relatively equal, 6.1 and 5.7 respectively (2013).

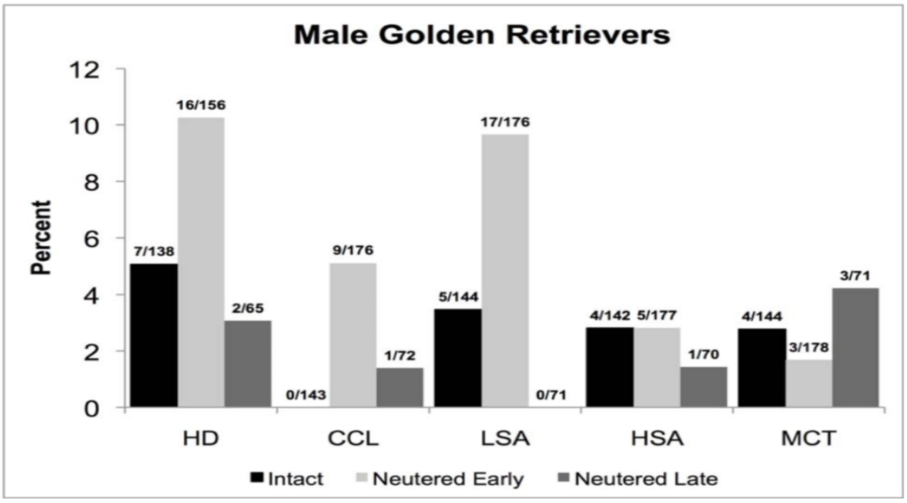

Figure 4: Percentages and number of cases of intact, early neutered and late neutered male Golden Retrievers aged between 1-8 years of age diagnosed with hip dysplasia (HD), cranial cruciate ligament tear (CCL), lymphosarcoma (LSA), hemangiosarcoma (HSA), and/or mast cell tumor (MCT) (Torres de la Riva *et al.*, 2013).

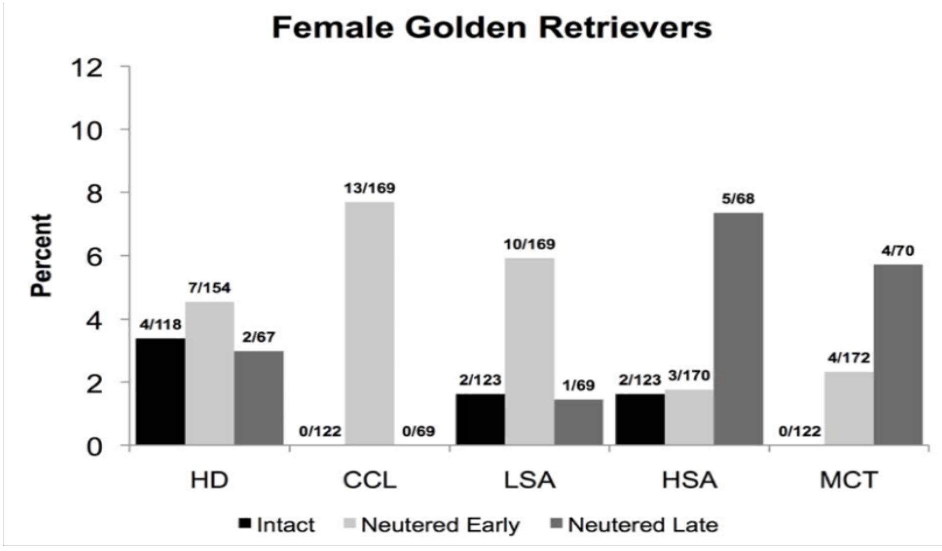

Figure 5: Percentages and number of cases of intact, early neutered and late neutered female Golden Retrievers aged between 1–8 years of age diagnosed with hip dysplasia (HD), cranial cruciate ligament tear (CCL), lymphosarcoma (LSA), hemangiosarcoma (HSA), and/or mast cell tumor (MCT) (Torres de la Riva *et al*., 2013).

4.3 Elbow Dysplasia

Another commonly diagnosed orthopaedic condition, particularly in medium and large breed dogs, is elbow dysplasia (ED) (James *et al.,* 2020). It is a hereditary disease, and its phenotypic development is influenced by numerous environmental factors (Lavrijsen *et al.,* 2014). It is a severe condition and can significantly impact the dog's welfare and quality of life (James *et al.,* 2020). ED occurs as a result of developmental disorders that result in malformation of the joint. Secondary to this, malformation conditions such as osteoarthrosis and arthrosis and degenerative joint disease occur. These secondary processes are what cause symptoms in the animals. Four main subclasses of conditions can characterise ED. These are osteochondrosis on the medial aspect of the humoral condyle, medial coronoid process fragmentation, ununited anconeal process and incongruity of the elbow joint (James *et al.,* 2020). These all result in the same outcome of lameness, joint pain and reduced range of movement of the joint (O'Neill *et al.,* 2020).

ED is mainly seen during or shortly after the rapid period of growth in young animals. It has been demonstrated that the larger a dog is, the more at risk it is for developing the disease. This could be explained by the fact that different breeds have different growth rates, and even within the same breed, the different sexes have different growth rates (Lavrijsen *et al.,* 2014).

Neutering has been one of the most frequently identified risk factors. A study was conducted in England used data from the VetCompass database to analyse 455,069 dogs from 304 Veterinary Clinics across the UK (O'Neill *et al.,* 2020). When the odds ratio for the prevalence of ED in neutered dogs was calculated, it was seen that neutered dogs had 1.69 odds of developing ED than dogs left intact (O'Neill *et al.,* 2020). Of the total population suffering from the condition, it was seen that 75% of all dogs diagnosed were neutered (O'Neill *et al*., 2020).

Another study carried out on German Shepherds once again showed a correlation between neutering and ED. In this study, the condition's occurrence was further analysed to examine if the timing of neutering was influential. The hospital records of the University of California Davis, Veterinary Teaching Hospital, were analysed, and 1170 dogs were chosen to make up the population of the study (Hart *et al.,* 2016). The neutering status was broken down into five categories, intact dogs, dogs neutered before 6 months of age, dogs neutered between 6-12 months of age, dogs neutered between 12-23 months, and dogs neutered between 1-8 years (Hart *et al*., 2016). Within the male group of dogs, the prevalence of ED in intact dogs was 2%. Dogs neutered before 6 months of age had a 4.4% risk of occurrence of ED, and dogs neutered between 6-11 months had a 5.3% occurrence of ED (Hart *et al.*, 2016).

The condition was much less prevalent in female dogs. It affected less than 1% of intact female dogs, and there was no occurrence in neutered dogs (Hart *et al.,* 2016). From this study, it is clear that gonadectomy has a significant role in the development of this condition in male dogs neutered early.

Similar results were seen in another study carried by Hart *et al.* on the effects of neutering on a cohort of Golden and Labrador Retrievers. The records of 1015 Golden Retrievers and 1500 Labrador Retrievers were chosen from the Veterinary Medical Database for the study. Again, the neutered dogs were divided into four groups, the dogs neutered before 6 months of age, between 6-11 months of age, at 1 year of age or between 2-8 years of age (2014). Of the Golden Retrievers analysed, neutering did not affect the risk of ED. The condition's prevalence was equal in both neutered and intact animals (2014). This was credited to breed-specific differences in the sensitivity of the growth plates to the sex hormones. Regarding the Labrador Retrievers again in females, the prevalence of ED was the same in both neutered and intact dogs. However, neutering male dogs of this breed before 6 months significantly increased the risk of ED. Additionally, male dogs neutered in the 2- 8-year bracket had a 2% increased risk of ED compared to intact dogs (2014). See Table 3.

Upon analysis of the three studies cited above, it is evident that there is a correlation between the early neutering of male dogs and the development of ED. From the research above, female dogs do not seem to be as affected by the condition irrespective of their neutering status. From the cited literature, it has been proven that ED is a hereditary disorder, thus in certain breeds, neutering of male dogs and the timing significantly impacts the probability of developing the condition. However, in other breeds who are not genetically susceptible to the condition, such as the Golden Retriever, the neutering status appears to be irrelevant in the development of ED.

It is known that neutering, through its role in obesity, can impact the onset of ED irrespective of the effect of the sex steroids on the growth plates and this was taken into consideration in both of these studies. In the study carried out by Hart *et al.* on the cohort of German Shepherds, the information analysed showed that the dogs who did develop this condition had a median BCS of 5, which is what is recommended. It was also shown that within the male dog group, the dogs that developed ED and the dogs that did not both had the same BCS of 5.0 (2016). This would suggest that the development of ED is linked to the early removal of the sex hormones, which results in delayed closure of the epiphyseal plates and the increased length of the long bones, which causes misalignment of bones in the joint. (2016).

In the third study referenced above that was carried out by *Hart et al.* on Golden and Labrador Retrievers, once again, the BCS and the occurrence of ED was taken into account. In this study, the BCS score of neutered dogs who suffer from the disease was notably higher than those intact dogs with ED. There was a 1.5 difference in the BCS of neutered and intact male Labrador Retrievers with ED (2014). This would suggest that the occurrence of obesity that results from neutering could play a more prominent role in contributing to the development of ED in Labradors than in other breeds (2014).

Table 3: The occurrence of joint disorders in a group of male and female Labrador Retrievers aged between 1- 8 years of age. . The number of cases are shown over the number in the pool. Percentages are given in brackets. The numbers in bold are used to indicate if the occurrence is significantly above that of intact dogs (Hart *et al*., 2014).

	HD	CCL	ED
Male <6 months	0/48 (0)	**4/53 (7.55)**	**2/48 (4.17)**
Male 6–11 months	1/68 (1.47)	2/72 (2.78)	0/67 (0)
Male 1 year	1/50 (2.00)	1/52 (1.92)	0/49 (0)
Male 2–8 years	0/92 (0)	0/93 (0)	**2/93 (2.15)**
Male Intact	9/528 (1.7)	12/531 (2.26)	3/525 (0.57)
Female <6 months	**3/56 (5.36)**	3/59 (5.08)	1/57 (1.75)
Female 6–11 months	**5/99 (5.05)**	5/101 (4.95)	0/103 (0)
Female 1 year	**2/47 (4.26)**	0/50 (0)	0/50 (0)
Female 2–8 years	0/131 (0)	1/128 (0.78)	0/132 (0)
Female Intact	6/345 (1.74)	8/343 (2.33)	4/343 (1.17)

4.4 PATELLAR LUXATION

Patellar luxation is one of the most commonly diagnosed orthopaedic conditions in dogs. It became a recognised condition over 50 years ago and can result in lameness, pain and osteoarthritis in the animal, severely impacting the quality of life (O'Neill *et al.,* 2016).

There are two clinically recognised forms of patellar luxation, medial and lateral. Medial luxation is the most commonly diagnosed of the two and is ten times more prevalent in miniature and toy breed dogs than their large breed counterparts. Following many years of research, it has been proven that Miniature and Toy Poodles, Yorkshire Terriers, Pomeranians, Pekingese, Chihuahuas and Boston Terriers are predisposed to this condition. This is due to defective development of the quadriceps muscle mechanism. Medial Patellar luxation has an equal probability of developing in either hind leg, and in 50% of diagnosed cases occurs bilaterally.

In contrast, lateral luxation occurs less frequently and is more prevalent in large breed dogs. It is diagnosed most frequently in young giant breed dogs or older small breed dogs. 50% of all diagnosed cases of lateral luxation are in large breeds (Roush, 1993).

A grading system has been established to categorise the severity of the luxation in individual animals. It operates on a scale of 1- 4. See Table 4. In Grade 1 and 2, the luxation is reversible, and in Grade 3 and 4, the luxation is permanent (Roush, 1993).

Table 4: The Grades of Patella Luxation (Roush, 1993)**.**

Grade 1	Patella can be manually luxated but returns to normal position when released.
Grade 2	Patella luxates with stifle flexion or on manual manipulation and remains luxated until stifle extension or manual replacement occurs.
Grade 3	Patella luxated continually. Patella can be manually replaced but will reluxate spontaneously when manual pressure is removed.
Grade 4	Patella luxated continually and cannot be manually replaced.

A study was carried out in England where the records of 210,824 dogs attending 119 Veterinary Surgeries were analysed to uncover further the prevalence, risk factors and clinical management of dogs diagnosed with patellar luxation (O'Neill *et al.,* 2016). The data was collected from the VetCompass Programme database. It was calculated that patellar luxation occurred in 1.3% of the population (O'Neill *et al*., 2016).

Of the dogs analysed, it was seen that female dogs were overrepresented with this condition. The results of this study showed that female dogs had 1.3 times higher odds of being diagnosed with the condition than male dogs (O'Neill *et al.,* 2016). This is consistent with the findings of other similar studies, such as the one carried out by Lavrijsen *et al.* on Dutch Flat-Coated Retrievers (2013). It was proven in an experimental study carried out on fourteen beagles by Gustaffson *et al*. that injections of estradiol benzoate resulted in shallow trochlear grooves, which is a primary cause for the development of patellar luxation (1969). Additionally, the effect of estradiol benzoate in reducing the thickness of the articular cartilage in the stifle joint was demonstrated (1969). These two findings explain the increased prevalence of patellar luxation in females.

The study carried out by O' Neill *et al.* also showed a correlation between neutering and patellar luxation. 88% of the dogs diagnosed were neutered. It was calculated that neutered dogs had 2.4 times the odds of having patellar luxation than dogs left intact. These results correlated with another study on small and miniature breed dogs in Austria, which found that neutered dogs had 3.1 higher odds of developing patellar luxation than intact dogs (2016).

While there is a proven link between neutering and this orthopaedic condition, the precise mechanism remains unknown. Neutering and sex steroid deficiency has been shown to cause other conditions in the stifle (O'Neill *et al.,* 2016). The lack of sex steroids has been shown to cause the overactivation of LH receptors in the connective tissue and disturbances in bone growth due to their effect on the epiphysis. These effects could additionally cause patellar luxation.

Studies carried out more recently have been documenting the proportion of neutered and intact dogs diagnosed with the disease, which was not done in the past. In time with more research, the exact mechanism will be discovered, as there is undoubtedly a statistical connection (Holt, 2017).

Upon analysis of the literature on this topic, it is evident that female dogs are more susceptible to this condition, and this is because of the effect of estrogen. Estrogen, as discussed earlier, has been proven to play a role in the development of another disease of the stifle CCLR. It is also clear that neutered dogs are more susceptible to the condition than intact dogs. However, as stated earlier, research into the exact correlation between neutering and patellar luxation is ongoing as is whether the age of neutering impacts the onset of this condition.

In the study carried out by O' Neill *et al.* referenced above, they did look at obesity as a possible predisposing condition for patellar luxation. However, using a multivariable method, the study demonstrated that the dogs most predisposed to the condition were of a smaller size and lower bodyweight than breed recommendations (2016).

4.5 INTERVERTEBRAL DISC DISEASE

Intervertebral disc disease (IVDD) is used to describe any lesion that affects the intervertebral disc (Fenn *et al.,* 2020). Between the 1940s and 1950s, Hansen and Olsson fundamentally separated IVDD into two distinct classes based on their histopathological features. This Hansen Type 1 and Hansen Type 2 herniations system of classification is still in use in clinical veterinary practice to this day (Fenn *et al.,* 2020). In both types, the mechanical functioning of the IVD unit fails due to herniation. Fissures on the annulus fibrosis and endplate sclerosis are seen. IVDD is the most commonly diagnosed cause of spinal cord injury (Fenn *et al.,* 2020).

Hansen Type 1 herniation is used to describe chondroid metaplasia degeneration. It is distinguishable by early-onset progressive dehydration and calcification of the nucleus pulposus. The result is the extrusion of the calcified nucleus pulposus into the vertebral canal through a ruptured annulus fibrosus. It has been proven that this type of IVDD is prevalent in chondrodystrophic breeds and that chondrodystrophic breeds are predisposed to it. (Fenn *et al.,* 2020). The reason for this genetic predisposition has been proven to be linked to the dog chromosome 12 locus, which is thought to support intervertebral disc calcification (Dickinson & Bannasch, 2020).

Hansen Type 2 IVD disease, herniation or protrusion is a chronic condition. It occurs in ageing non- chondrodystrophic dogs, usually over seven years of age. The disc undergoes fibroid metaplasia degeneration which occurs at a slow rate. The proportion of collagen in the disc increases, and the notochordal cells become fibrocyte-like. This ultimately results in protrusions on the annulus fibrosis surface, which cause the movement of the dorsal longitudinal ligament from its normal positioning. This causes spinal compression (Fenn *et al.,* 2020).

The dachshund is one of the chondrodystrophic breeds known to be predisposed to Type 1 IVDH (Dorn & Seath, 2018). A large-scale study involving 1,964 dachshunds suffering from IVDD was established by Dorn and Seath to investigate the correlation between neutering and IVDD. This was the first large-scale study of its kind. The aim was first to establish if gonadectomy increased the probability of a dog developing this disease and secondly to see if the timing of the gonadectomy had an influence. The data was collected from the results of a questionnaire completed by dachshund owners. The survey

“Daschlife2015 The UK Dachshund Breed Council’s Back Disease (IVDD) and Lifestyle Survey” was distributed online using social media and via the online newsletter of the UK Dachshund Breed Council (2018). For the dogs in the age 3-10 years old age bracket, the prevalence of IVDD in early neutered dogs, late neutered and intact dogs for each gender were compared. Early neutering was considered to be before 12 months of age, and late neutering was considered to be after 12 months (2018).

Of the 1964 dachshunds included in the study, 54% of all male dogs were neutered, and within that group, 64% were neutered early, and the remaining 36% were neutered after 12 months of age. With regards to the females, 58% of all bitches were neutered. 49% were neutered before 12 months of age, and 51% were neutered after 12 months (2018).

The study showed that neutering has a significant impact on the development of this condition, particularly early neutering. Both male and female dogs neutered before 12 months of age had a substantially higher probability of IVDH than intact dogs. Female dogs neutered at any age were at a higher risk of developing IVDH than their intact female counterparts (2018).

Following analysis, the results revealed that early neutered females were twice as likely to develop IVDH than intact bitches. Bitches neutered after 12 months of age were also shown to have a considerably higher risk of developing IVDH than entire female dogs. When the incidence of the condition between early and late neutered females was compared, it was illustrated that early neutered dogs were the group more at risk (2018).

Within the male dogs, the incidence of IVDH was notably increased in early neutered dogs compared to intact dogs. However, the risk of IVDH in dogs neutered after 12 months and intact dogs was relatively equal (2018). See Table 5.

The effect of the gonadal hormones on IVDD is relatively unexplored in dogs of both sexes. In human medicine, research has been carried out to show the effect of estrogen on the intervertebral discs in peri-and postmenopausal women. Reduced lower back pain has been seen in perimenopausal women given injections of estrogen. In postmenopausal women, an increase in intervertebral disc height has been shown following estrogen injections. This could explain the effect, but further research into the area is required (2018).

Studies have been carried out on the role of the sex hormone receptors on the chondrocytes in the articular cartilage. Estrogen receptors have been identified on the discus annulus cells in humans and nucleus pulposus cells in rats. Androgen receptors have additionally been identified on the disc cells of humans. It has been shown that the proliferation of the human disc annulus cells is facilitated by estrogen. Furthermore, estrogen assists the rat nucleus pulpous cells in the increased deposition of collagen 11, aggrecan and glycosaminoglycan. It has also been hypothesised that estrogen may have a protective role. It has been proven in rats that estrogen inhibits the calcification of the endplates cartilage, reduces oxidative damage in the cartilage cells, decreases the apoptosis of annulus fibrosus cells and defends the pulpous nucleus cells against early senescence induced by TNF-α. The addition of testosterone to the human disc cells increased the expression of extracellular matrix proteins, particularly Type 2 collagen and aggrecan (2018).

Another theory is that via their role in the behaviour of cells, on the expression of genes and through their influence on the chemical composition of the nucleus pulposus and the annulus fibrosus matrix, sex steroids influence the susceptibility of the canine discs to damage. These effects occur over time, which would explain why following gonadectomy and the sudden removal of the sex steroids in young dogs, the effects on the disc are so severe. The mechanism of action of the sex hormones is thought to be connected to the cytokine pathway and dependent on the concentration of the sex hormones (2018).

A study carried out by Hart *et al.* revealed similar results in the correlation between neutering and IVDH in another chondrodystrophic dog, the Corgi. Of the 240 dogs of this breed analysed, IVDH was reported in 3% of intact males and 8% of intact females. In male dogs neutered before six months of age, the prevalence of IVDH reached 18% (2020).

Upon analysis of the above literature, it is my opinion that neutering dogs increases the occurrence of this condition. IVDD is notably more prevalent in neutered than intact dogs. There are gender differences in the effect of neutering on this condition. In both male and female dogs, early neutering resulted in a significantly increased occurrence of the condition compared to intact dogs. However, while in male dogs, the prevalence of IVDD is relatively equal in dogs neutered after 12 months of age and those left intact, in bitches, late neutering results in an increased occurrence of IVDD in comparison to intact female dogs. As I stated previously, the study carried out by Dorn and Seath was the first large scale study carried out on the link between IVDD and neutering, and it was carried out exclusively on the dachshund. A link between neutering and IVDD was also seen in the Corgi in a study carried out by Hart et al. It is therefore evident that there is a strong correlation, and as more research is done into this area, more information will become available.

As with all orthopaedic conditions, obesity as a predisposing factor in IVDD must be considered. However, as the information collected in the study carried out by Doth and Seath was from a questionnaire and the owners were determining the BCS of their own animal, the results cannot be considered completely objective, and thus, obesity was excluded as a variable in the study (2018).

Table 5: Incidence rate and risk of IVDD in a group of dachshunds aged between 36 and 120 months of age (Dorn & Seath, 2018)**.**

Sex and neuter status	DYAR	Incidence (No. of cases)	Incidence per 1000 DYAR	Mean risk of IVDH per DYAR	RR compared with entire animals	RR compared with late-neutered animals
Entire bitches	838	45	54	5%	1	/
Bitches neutered < 12 months	456	52	114	11%	**2.12 (1.44–3.11)**	1.37 (0.93–2.01)
Bitches neutered > 12 months[a]	554	46	83	8%	**1.55 (1.04–2.30)**	1
All neutered bitches	1010	98	97	10%	**1.81 (1.28–2.54)**	/
Entire males	936	55	58.7	6%	1	/
Males neutered < 12 months	574	52	90.6	9%	**1.54 (1.07–2.22)**	1.38 (0.96–1.99)
Males neutered > 12 months[a]	365	24	65.8	7%	1.12 (0.7–1.78)	1
All neutered males	940	76	80.9	8%	1.38 (0.98–1.93)	/

Figures shown in bold are considered statistically significant at the 95% confidence level
95% confidence intervals are shown in brackets
DYAR Dog years at risk
RR Risk ratio
[a]Bitches and dogs neutered > 12 months are "late neutered"

4.6 OSTEOSARCOMAS

In addition to orthopaedic conditions, neutering is strongly associated with the development of several types of cancer. One of these cancers is osteosarcoma. Osteosarcoma is a fast developing and painful tumour that develops in the bone. Certain dogs are predisposed to the development of this neoplasm. These mainly include large breed dogs that have heavy body masses and extended long bones. A genetic predisposition in some breeds has been demonstrated, such as in the Rottweiler (Edmunds *et al*., 2021).

Osteosarcoma predominantly occurs in two anatomical locations. In large breed dogs, osteosarcoma is prevalent in the appendicular skeleton. 95% of all cases diagnosed in large breed dogs are located in the pelvis and the limb. While only a small proportion of small breed dogs are affected by osteosarcoma, 65% of the cases are associated with neoplasms in the axial skeleton, i.e. the bones of the skull, the cervical vertebrae, the vertebrae of the spine, the ribs and the sternum (Edmunds *et al.,* 2021).

Osteosarcoma is a severe condition. Although surgical treatment and the removal of the affected limb is possible, in most cases, early hematogenous spread to the lungs has already occurred. 90% of cases diagnosed have been demonstrated to have microscopic metastasis at the time of the initial diagnosis, even if gross lesions are not visible. Following surgery and chemotherapy, the median survival rate for one year is 45-50% (Edmunds *et al.,* 2021).

It has been shown that neutering a dog can significantly increase the risk of osteosarcoma. It is thought that the reason neutered large breed dogs are considerably more affected is due to the more significant impact caused by the osteoblast maturation disruption. A study conducted on German and Australian shepherd dogs showed that the prevalence of osteosarcoma within both breeds increased in neutered dogs of both sexes (Oberbauer *et al.,* 2019).

In contrast, additional studies on dog breeds predisposed to osteosarcoma showed that while neutering increased the risk of neoplasm in female dogs, the effect neutering had on the risk of males was insignificant. Furthermore, studies have shown that neutering male hound dogs such as the Basset Hound and Afghan hounds decreases their risk of developing this cancer. The prevalence of osteosarcoma in neutered dogs of this group is less than in intact male dogs. In contrast, other studies on dog breeds predisposed to osteosarcoma showed that while neutering increased the risk of neoplasm in female dogs, the effect neutering had on the risk for males was insignificant. However, the opposite effect was seen for female hound dogs whose risk of osteosarcoma was shown to increase following spaying (Oberbauer *et al.,* 2019).

A study carried out by Cooley *et al*. further broke down the role of neutering in the development of osteosarcomas to see if it was influenced by early or late gonadectomy and if the sex of the dog was significant. The study used the data of a group of 683 Rottweilers. Rottweilers were chosen as they are one of the breeds genetically predisposed to this condition (2002).

Upon analysis of the results, it was shown that there are significant variations in the prevalence of the condition depending on the age at gonadectomy. The dogs that were neutered early, i.e., before one year of age, had a significantly increased risk of osteosarcoma. Both male and female dogs who were neutered early had a one in four-lifetime risk for developing osteosarcoma in later life. Furthermore, it was calculated that for each extra month the animal was left intact, the risk of osteosarcoma decreased by 1.4% (2002). See Figure 6.

Further variations were seen among the sexes. The prevalence of osteosarcomas in male dogs neutered before one year of age was nearly four times higher than the prevalence in sexually intact male dog group. See Figure 7. In relation to female dogs, the incidence rate was three times higher when the same two groups were compared (2002).

	Dogs with bone sarcoma (no.)	Dogs without bone sarcoma (no.)	Total dog-months	Bone sarcoma incidence rate (95% CI)[a]	RR (95% CI)	*P*
Total population	86	597	71,004	12.1 (9.6–14.7)		
Gender						
Male	35	259	30,228	11.6 (7.8–15.4)	1.0	
Female	51	338	40,776	12.5 (9.1–15.9)	1.1 (0.7–1.7)	0.74
Male gonadal exposure subgroup						
Castrated before 1 yr of age	9	25	3,168	28.4 (9.8–47.0)	3.8 (1.5–9.2)	0.002
Castrated 1–3.5 yr of age	8	57	6,228	12.8 (3.9–21.8)	1.7 (0.7–4.3)	0.31
Castrated after 3.5 yr of age	8	57	7,632	10.5 (3.3–17.8)	1.4 (0.6–3.5)	0.48
Sexually intact	10	120	13,212	7.6 (2.9–12.3) *P* trend = 0.008	1.0	
Female gonadal exposure subgroup						
Spayed before 1 yr of age	18	57	7,176	25.1 (13.5–36.7)	3.1 (1.1–8.3)	0.02
Spayed 1–5 yr of age	14	108	12,612	11.1 (5.3–16.9)	1.4 (0.5–3.8)	0.63
Spayed after 5 yr of age	14	108	14,856	9.4 (4.5–14.3)	1.2 (0.4–3.2)	1.00
Sexually intact	5	64	6,144	8.1 (1.0–15.3) *P* trend = 0.006	1.0	

Figure 6: Lifetime exposure to gonadal hormones and the risk of bone sarcoma in a group of 683 Rottweiler dogs (Cooley *et al*., 2002)**.**

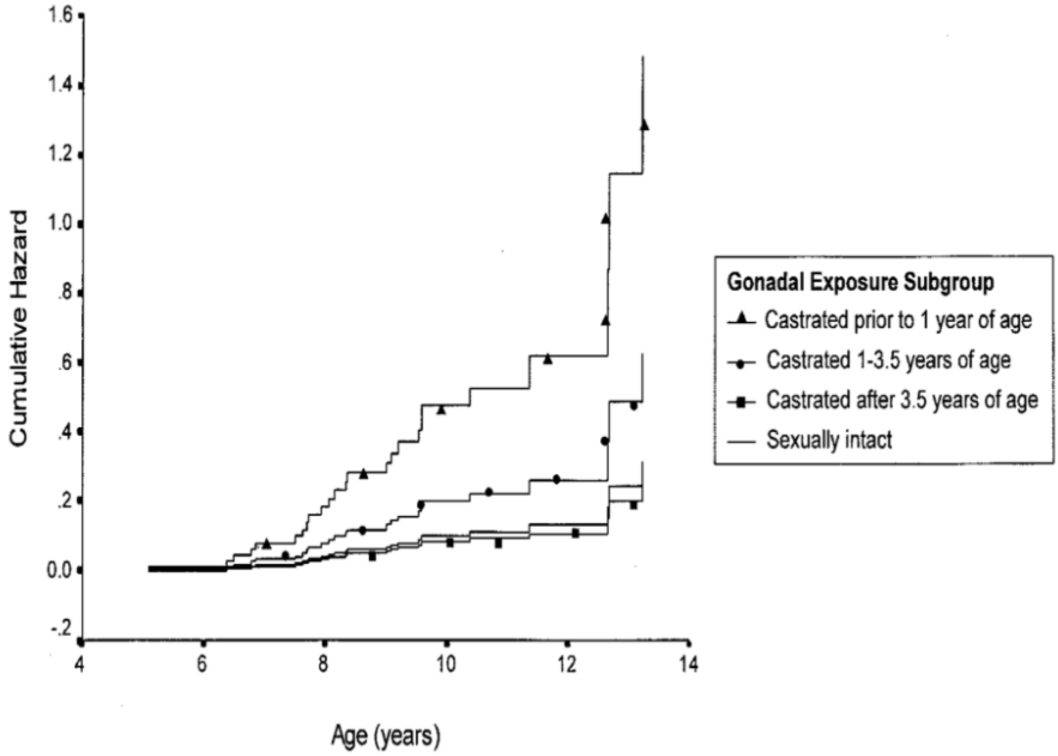

Figure 7: Multivariate hazard functions for the risk of bone sarcoma in male Rottweiler dogs according to the exposure of gonadal hormones. (Cooley *et al.,* 2002)**.**

From the evidence seen above, it is clear that gonadectomy results in the increased occurrence of osteosarcoma in spayed female dogs. However, the effect in male dogs differs between various breeds. The reason why females are more susceptible is thought to be due to the removal of estrogen. The typical differentiation of the osteoblastic cells is influenced by estrogen, and osteosarcoma is related to rapid bone growth. After spaying, the primary source of estrogen is removed. The lack of estrogen stimulates, through increased proliferation and increased activity, the expansion of osteoblasts. The osteoblastic expansion is thought to contribute to the development of osteosarcoma in large breed female dogs who typically undergo rapid growth and highly active osteoblast cells (Oberbauer *et al.,* 2019).

Neutering is also thought to influence the development of cancers by suppressing the production of specific T-cells. Suppressor and cytotoxic T-cells have been shown to play a critical role in immune surveillance, particularly in the destruction of cancer cells. Studies on male cattle, rabbits and guinea pigs, and male and female mice have shown that gonadectomy, through increasing the size of the thymus gland, decreases the number of thymocytes that produce these two T-cells (Oberbauer *et al.,* 2019).

METHOD

When I initially started the research for my literature review, I found that there was a multitude of articles written and studies carried out on the topic. The main objective of my thesis was to analyse and review the correlation of the neuter status and clinical manifestations of specific orthopaedic conditions. In addition to this, I set out to prove that the age of neutering and the sex of the dog was significant. I chose six orthopaedic conditions as the focus of my literature review. Many more orthopaedic conditions that correlate with neutering exist. I specifically chose CCLR, HD, ED, Patellar Luxation, IVDD and osteosarcomas to review as the literature on these conditions met the selection criteria, I needed to prove my objectives.

For each orthopaedic condition reviewed, I ensured that there was adequate information available to support my objectives. This entailed ensuring that numerous articles written by a range of authors existed that proved the correlation between neutering and the specific orthopaedic condition analysed. I made sure to include at least two studies for each condition, both of which positively correlated with my objectives.

Additionally, I ensured that there was a proven link between neutering and the orthopaedic condition in more than one specific breed. For example, in my review of CCLR, I first used an article that proved that the prevalence of CCLR increased in neutered dogs. This study carried out by Witseberger. *et al.* used data collected from 53 breeds, and for the dog breed to be included in the study, data from at least 4,000 individuals from each breed had to be evaluated (2008).

It was important that in each article I reviewed, the sample size was large enough to be representative of the population. The Witsberger *et al.* study, which I used to analyse the prevalence of CCLR, had a sample size of 1,243,681 dogs (2008). Another study which I based my literature review upon, carried out by Hart *et al.* on the occurrence of CCLR and ED on German Shepherds and used data collected from 1170 dogs. The population size was important in my review as it was important that the results collected, which I based my analysis on, were statistically significant (2016).

Diversity was another critical factor that needed to be met in my selection criteria. It was important that in each study, male and female dogs were represented equally within the population. In the Hart *et al.* study, which compared Labrador and Golden Retrievers of the

1,015 Golden Retrievers analysed, 543 were male, and 472 were female. Within the population of 1,500 Labrador Retrievers, 808 were male, and 692 were female (2014). In the van Hagen study, which I used to analyse the occurrence of HD in a population of 1,863 boxers comprised of 53.3% female dogs and 46.7% male dogs (2005).

Another crucial factor when deciding what articles to select for my literature review was that the population was divided into intact, early neutered and late neutered groups for analysis and that each group was equally represented. In the Dorn and Seath study on IVDD in Dachshunds, of the 1013 male dogs analysed, 54% were neutered. Of the 54%, 64% of the male dogs were neutered before 12 months, and 36% were neutered at 12 months or later. 58% of the 951 female dogs analysed were neutered. 49% were neutered before 12 months of age, and 51% were neutered at 12 months of age or later (2018). In the Torres de la Riva *et al*. study on Golden Retrievers, of the 359 male dogs analysed, 145 were intact, 168 were neutered before 12 months of age, and 72 were neutered after 12 months of age. Of the 364 female dogs, 122 were intact, 172 were neutered before 12 months of age, and 70 were neutered after 12 months (2013).

Finally, for each of the studies I used, I made sure that other predisposing factors that may have influenced the occurrence of the orthopaedic condition were taken into account. Such predisposing factors included age and obesity.

Concerning age, the studies needed to include data from dogs of a wide range of ages, not just data from older dogs. As dogs get older joint disorders are seen more commonly, and the occurrence of orthopaedic conditions needed to be shown in dogs of all ages, not just geriatric dogs. The Torres de la Riva *et al.* study used data from Golden Retrievers who ranged from 1-8 years. No data from dogs under 12 months of age or dogs older than 9 years of age were used. In this study, all dogs chosen had to have a recorded date of birth (2013). The Hart *et al.* study on German Shepherds collected and analysed data from dogs up to 8 years of age. Data from dogs over eight years old were not included in the study (2016).

Obesity is one of the major predisposing factors for orthopaedic conditions, and it has been proven that neutering predisposes dogs to obesity. Two mechanisms are thought to be responsible for the link between obesity and neutering. These mechanisms are increased appetite and a diminished metabolic rate (Kutzler, 2020). In my selection criteria, the studies needed to take this connection into account when formulating their results. In the Hart *et al.* study on German Shepherds, the median BCS score of the neutered dogs suffering from the condition was calculated and compared to the BCS of the neutered dogs who did not suffer from the condition (2016). This showed an increased prevalence of joint disorders in neutered dogs irrespective of their body weight. This was also considered in the Torres de la Riva *et al.* study and the Hart *et al*. study on Labrador and Golden Retrievers. The 2016 and 2020 studies carried out by O' Neill *et al.* calculated a bodyweight relative to breed mean variable and used this to analyse the results. This entailed calculating the mean adult bodyweight specific to breed and sex and using this variable to classify individual dogs in the study population as below, equal or above the mean (2020).

RESULTS

The results of the systematic literature review of the six specific orthopaedic conditions were as follows. Each of the six orthopaedic conditions reviewed will be discussed.

Cranial Cruciate Ligament Rupture

To analyse the occurrence of CCLR and consider if the neutering status, sex of the dog, and age at neutering were significant, I based my literature review around three studies.

Initially, to examine if CCLR was a common orthopaedic condition in daily veterinary clinical practice, I looked at the study carried out by Witsberger *et al*. in 2008, which used a population of dogs chosen without bias to sex, age and breed. This study analysed the occurrence of CCLR in a population of 1,243,681 dogs and found that 31,698 suffered from CCLR. It had a prevalence of 2.55% in the population studied, proving that it is a relatively common condition (2008).

Next, to examine if CCLR is influenced by the age at neutering and the sex of the dog, I focused on two major studies. Both were based on two popular breeds the Golden Retriever and the German Shepherd. The Torres de la Riva *et al.* study was carried out on a group of 759 Golden Retrievers and found that in both male and female dogs who were left intact and neutered after 12 months of age, CCLR did not occur. In the dogs neutered before 12 months of age, the prevalence of CCLR was 5.1% in male dogs and 7.7% in bitches (2013).

The second study-was carried out by Hart *et al.* on a group of 1170 German Shepherds. The incidence of CCLR in both intact male and female dogs was less than 1%. Within the population of male dogs, 12.5% neutered before six months of age, and 8.3% that were neutered between 6-11 months were diagnosed with CCLR (2016). In the female dogs spayed before six months of age, the occurrence of CCLR was 4.6%, and 8.3% in the bitches spayed between 6-11 months of age (2016). The results of these studies proved firstly that the incidence of CCLR in unneutered male and female dogs is negligible. However in both sexes, early neutering of dog's results in an increased susceptibility to CCLR. Secondly it proved that there are breed-specific differences in the effect of neutering and the occurrence of CCLR. In Golden Retrievers, neutering results in an increased probability of CCLR in females, and in German Shepherds, it is early neutered male dogs who are predominantly affected.

Hip Dysplasia

For my review of HD I focused my literature research and analysis around two core studies. First, to prove that HD is influenced by gonadectomy, I used the study carried out on 1,863 boxers by von Hagen *et al.* This study showed that neutered dogs were 1.5 times more likely to develop HD than their intact counterparts (2005). I used the 2013 Torres de la Riva *et al.* study to prove the link between the sex of the dog and the age at neutering and the prevalence of this orthopaedic condition. The study results showed that HD affected 10.3% of male dogs neutered before 12 months of age and that only affected 5.1% of the male dogs left intact. It also showed that of the female Golden Retrievers analysed, the timing of gonadectomy was not significant. The study also calculated the mean age for the onset of the disorder in males which was; in intact male dogs 4.4 years, in early-neutered dogs 3.6 years and late neutered dogs 4.7 years (2013). These studies demonstrated that gonadectomy is a predisposing factor in developing HD and that male dogs are significantly more affected than female dogs. The age of neutering impacts the susceptibility and the development of the condition.

Elbow Dysplasia

I analysed three critical studies in my systematic literature review of ED. The study carried by O' Neill *et al*. in 2020, used data from 455,069 dogs living across the UK and calculated the odds ratio for ED in neutered dogs (2020). The study results showed that neutered dogs had a 1.69 higher probability of developing ED compared to intact dogs (2020). This study proved that gonadectomy is a predisposing factor in the development of ED.

To demonstrate the correlation between sex and age of neutering and ED, I used two breed-specific studies. The study carried out by Hart *et al.* on 1170 German Shepherds showed that the prevalence of ED in intact male dogs was 2%. Male dogs neutered before six months of age had a 4.4% risk of developing ED, and dogs neutered between 6-11 months had a 5.3% risk of developing ED (2016). In comparison, less than 1% of intact female dogs were diagnosed with ED, and no female neutered dogs were affected (2016). Similar results were seen in the study carried out by Hart *et al.* on 1015 Golden Retrievers and 1500 Labrador Retrievers. (2014) Neutering did not affect the risk of ED in Golden Retrievers. The condition's prevalence was equal in both neutered and intact animals (2014). In female Labrador Retrievers, the prevalence of ED was the same in both neutered and intact dogs. However, male dogs neutered before six months showed significantly increased risk of ED. Additionally, male dogs neutered in the 2 to 8-year bracket had a 2% increased risk of ED compared to intact dogs (2014).

These two studies show that early neutering increases the occurrence of ED specifically in male dogs and that some dog breeds are more susceptible to ED than others.

Patellar Luxation

For my systematic literature review of patellar luxation, I used two studies to support my objectives. The study carried out by O'Neill *et al.* in 2016 on 210,824 dogs showed that the prevalence of patellar luxation was 1.3% within the population (2016). The results of this study also showed that female dogs were 1.3 times more likely to de diagnosed with this condition than male dogs, and that of the dogs diagnosed with patellar luxation 88% were neutered (2016). Additionally, O' Neill *et al.* calculated that neutered dogs had 2.4 times the odds of having patellar luxation compared to dogs left intact (2016). This study proved that one, neutering dogs increases the occurrence of patellar luxation and two, that females are more susceptible to patellar luxation in males.

Additionally, to support the result that bitches are more at risk of developing patellar luxation than male dogs, I used an experimental study by Gustafsson *et al.* on 14 Beagles in my literature review (1969). The results of this study showed that injections of estradiol benzoate resulted in shallow trochlear grooves and reduced thickness of the articular cartilage in the stifle joint (1969). These two findings explain the increased prevalence of patellar luxation in females.

Intervertebral Disc Disease

For the specific orthopaedic condition IVDD, I primarily focused my literature review on the study carried out by Dorn and Seath on the Dachshund. The data of 1,964 dachshunds was analysed, and it was seen that within the population, of the 54% of male dogs that were neutered, 64% were neutered before 12 months of age, and the remaining 36% were neutered after 12 months of age. With regards to the female dogs, 58% of all bitches were neutered. 49% were neutered early, and 51% were neutered after 12 months (2018). The results of the study showed that early neutered females were twice as likely to develop IVDH than intact bitches. Bitches neutered after 12 months of age were also shown to have a considerably higher risk of developing IVDH than intact female dogs. When the incidence of the condition between early and late neutered females was compared, it was seen that early neutered dogs were more at risk (2018). Upon analysis of the male dogs included in the study, the results demonstrated that in early neutered dogs, the incidence of IVDH was notably increased compared to intact dogs. However, the risk of IVDH in dogs neutered after 12 months and intact dogs was relatively equal (2018). This study showed that the condition is more prevalent in neutered dogs than intact dogs in both sexes. However, while in female dogs, early and late neutering resulted in a higher incidence of IVDD, in male dogs, only dogs that were neutered early had an increased susceptibility to developing IVDD. I supported these claims with a study carried out by Hart et al. on a study carried out on 35 specific dog breeds, one of which was the Corgi. Of the 240 dogs of this breed analysed, IVDH was reported in 3% of intact males and 8% of intact females. In male dogs neutered before six months of age, the prevalence of IVDH reached 18% (2020). This once again showed that early neutering of male dogs increases the occurrence of this condition.

Osteosarcomas

For the final orthopaedic condition, osteosarcomas, I primarily focused on the study carried out on 683 Rottweillers by Cooley *et al*. The results revealed that the dogs neutered early, i.e., before one year of age, had a significantly increased risk of osteosarcoma. Both male and female dogs who were neutered early had a one in four-lifetime risk for developing osteosarcoma in later life. Furthermore, it was calculated that for each extra month the animal was left intact, the risk of osteosarcoma decreased by 1.4% (2002). When the occurrence within the different sexes was analysed, it was seen that the prevalence of osteosarcomas in male dogs neutered before one year of age was nearly four times higher than the prevalence in the sexually intact male dog group. In female dogs, the incidence rate was three times higher when the same two groups were compared. This study demonstrated that neutering dogs increases their susceptibility to osteosarcomas in both sexes and that male dogs are marginally more susceptible to the condition than female dogs (2002).

The results of all of these studies supported my objectives which I outlined earlier in this paper. In each of the six specific orthopaedic conditions, it was seen that neutering dogs did increase their susceptibility to the development of these orthopaedic conditions. The results also showed that, in general, dogs who were neutered earlier had an increased occurrence of these conditions than dogs who were neutered later in life. Whether male or female dogs were more affected was variable and depended on each specific condition.

DISCUSSION

The studies used in my literature review comprised of three main categories. The first category analysed data from Veterinary Medical Databases. Data from dogs that had at least one record at a veterinary hospital between a specific time interval were chosen for the study population. The second category were questionnaire-based studies. Data was collected and analysed from questionnaires completed by individual dog owners. The final category of studies were experimental studies. Experimental tests and procedures were carried out on a group of animals, and the results were collected and analysed. Although I tried to collect data from a wide range of different sources, each study has its potential faults that may influence the results.

The majority of the studies I used were based on data collected from electronic Veterinary Medical Databases. These included the three studies carried out by Benjamin Hart *et al.,* the Torres de la Riva *et al*. study and the two O'Neill *et al.* studies. The advantage of these studies was that it is easy to generate a large sample size. The disadvantage was that some information that was a prerequisite for the animal to be included in the study was missing. In the Hart *et al.* study on German Shepherds, an increased proportion of intact compared to neutered dogs was included in the sample compared to what one would expect in the general population of dogs. Some of the records in the database did not have the age of neutering recorded, and even though an attempt was made to contact the referring veterinarian, in some cases, the age of neutering was unknown. In this case, the dogs were excluded from the study, resulting in an increased number of intact compared to neutered dogs (2016). In the Witseberger *et al.* study on HD and CCLR, the role of obesity as a predisposing factor was not considered as the data available from the Veterinary Medical Database on the BCS and bodyweight of the dogs included in the study was unreliable (2008).

The van Hagen *et al.* study on a cohort of boxers and the Dorn and Seath study on a cohort of dachshunds used data collected from owner-based questionnaires. In the van Hagen *et al.* study data was collected from each animal every six months (2005), while the Dorn and Seath study was based on data collected from one large scale questionnaire. An issue encountered with this type of study is that the data collected is from information given by the owner. In the Dorn and Seath study, obesity could not be used as a variable to examine the correlation of obesity and IVDD as the owner was the individual determining the BCS of their own pet, and thus their evaluation could be considered inaccurate. In this study, only 6.6% of the dogs were reported as being higher than average weight, which, when considered with the proportion of obese dogs within the general population in the UK of 24.3 -59.3%, seems very unlikely (2018).

Another issue encountered with both types of studies is that they only compare dogs clinically diagnosed with the specific orthopaedic condition to those who do not suffer from the condition. For example, in the van Hagen *et al.* study, only the dogs with hindlimb lameness were subsequently radiographed and CCLR seen on the radiographs were used in the study. In reality, dogs who do not suffer from hindlimb lameness could still have radiographically evident CCLR, but it is undiagnosed. If radiographs were taken at a fixed age in all dogs in a study population, the results would be more accurate; however, in this study and most others, this is unrealistic (2005).

The final form of study I used in this literature review were experimental studies. I used these to understand and analyse the effect of the hormone on the orthopaedic system and to explain why the prevalence of these specific orthopaedic conditions increased in neutered dogs. The Gustaffson *et al.* study used a group of 14 beagles to analyse the effect of estradiol on patellar luxation. While the sample size of the population was small, all precautions were taken to ensure the experiment was accurate. All the 14 dogs were born in the kennels of the Hospital for special surgery, all weaned at 6-8 weeks of age, and all fed the same commercial diet. The benefit of these studies is that all external factors that could influence the result are removed (1969).

The aim of my literature review was to assess if neutering dogs increases the occurrence of specific orthopaedic conditions and if the sex of the dog and the age of neutering play a role. I gathered as many sources as possible and used studies with significant population sizes and articles written by respected authors. The majority of the studies used were breed specific. Ideally, I would have analysed more studies focusing on dogs of two or more breeds or even crossbred dogs. As of now, very few articles like this have been published. This is a topic of great interest on which much research is being conducted and more articles will be produced in the near future.

CONCLUSION

My interest in Veterinary Medicine has always been small animals, and neutering is one of the most commonly performed procedures. As such, I think it is essential to know the risks as well as the benefits. Following my literature review, my professional opinion is that while there are significant benefits associated with neutering female dogs, in male dogs, it is questionable if the benefits of neutering outweigh the risks.

Neutering has an unrefutable role in the development of orthopaedic conditions through both direct and indirect effects. The direct effect of sex steroid removal is the delayed closure of the physeal plates of the bones and the overactivation of the LH receptors. In addition to this, neutering has a proven correlation with obesity. Obesity is one of the leading predisposing factors in the development of orthopaedic conditions. This creates a vicious cycle, and it becomes challenging to differentiate cause and effect.

The primary benefit of neutering in both sexes is clearly the role it plays in population control. In female dogs, gonadectomy plays a critical role in preventing pyometra and reduces the risk of malignant mammary gland tumours. While there are side effects to this procedure, such as the risk of developing urinary incontinence, obesity and the increased probability of developing orthopaedic conditions, in female dogs, these potentially life-prolonging benefits outweigh the risks. In male dogs, the benefits of neutering are negligible. The small number of benefits of neutering, such as reducing the risk of non-cancerous prostate diseases, are drastically outweighed by the increased risk of obesity and orthopaedic conditions associated with neutering.

Following this literature review, it would be my opinion that in the interest of the long-term health of the animal that male dogs should be left intact and that female dogs should be neutered. Furthermore, the age of neutering of the animal is crucial. While neutering at any age predisposes to orthopaedic conditions, the risk is significantly decreased if the dog is neutered after 12 months of age.

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ACKNOWLEDGEMENTS

First of all, I would like to thank my Thesis advisor, Dr. Linda Müller, for her support and guidance throughout the entire thesis writing process.

Secondly, I would like to thank my parents and brother for their constant encouragement, advice and unfaltering support. Without them, none of this would have been possible.

I would like to thank all of the Professors at the University of Veterinary Medicine for ensuring that we received such a high standard of education.

Finally, I would like to thank my friends both from home and from university for always being there to cheer me up whenever things got a bit too stressful!

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- *present the knowledge base of the University of Veterinary Medicine Budapest and its partners in a focussed way in order to improve the prestige of the Hungarian veterinary profession, and the competitiveness of the organizations in question;*
- *facilitate professional relations and collaboration;*
- *support open access.*

I hereby confirm that I am familiar with the content of the thesis entitled

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manifestations of orthopaedic disorders - systematic literature review .... written by ......IMOGEN WHITE......................................
(student name) which I deem suitable for submission and defence.

Date: Budapest, ...18...day.....11......month ...2021.year

DR LINDA MÜLLER....
.... Supervisor name and signature

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