Examining Differences in Blood Calcium, Phosphorus, and T4 Levels in Various Breeds of Canines: A Study on its Association Between the Age of Spay/Neutering and Osteopathic Diseases

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I. Personal Section

I've always had a keen interest in the field of veterinary medicine. Towards the beginning of my high school career, I discovered animal physical rehabilitation. I had realized there was this field completely devoted to the healing process for various animals' injuries and even used as a surgical alternative. As I explored this topic further, I wanted to do something about it, so I decided to sign up for the Science Research program during my Freshman year. Little did I know, this small interest of mine would flourish into a passion that I'd be pursuing as a career.

I knew that I wanted to delve into something within this field, but had practically no idea what I was going to specifically research for the next 3 years. I started off as a clueless Sophomore trying to analyze journal articles about integrating prosthetics into mice, all the way through contacting numerous authors from these journal articles with questions that were never successfully answered. I was starting to question this topic of interest until I was fortunate enough to acquire my first mentor, Carly Arbitman, at the Canine Rehab of NY. I began by shadowing her and the other canine therapists for a few weeks. Through my shadowing her, I was able to see her rehabilitate certain patients to take their first steps again. Seeing the owners' faces light up after seeing their dog get this second chance at life after being told they wouldn't be able to walk again was truly an inspiring and invaluable experience.

As I continued to work with Carly, my passion and desire to research within this field grew. She briefly mentioned one day that throughout all her years of working with these canines, she had noticed that almost none of them were intact, and a vast majority of them were neutered at a premature age. I kept what she said in mind, and I started to question why. These canines that she rehabilitated daily had some of the most severe diseases that very often resulted in paralysis or even death, and for some reason, there was a pattern of almost all of them having been neutered early on in life. Why? With not much knowledge on the topic of neutering and spaying, I continued to read and analyze numerous journal articles. I remember thinking that there was this pattern with humans. For example, women after menopause facing estrogen deficiency and osteoporosis, or men being twice as likely to develop prostate cancer after undergoing a vasectomy. As I continued to gradually gain my understanding, I learned that this

was an extremely controversial topic amongst pet owners, veterinarians, and no-kill animal shelters. Neutering/spaying at less than 1 year of age offers numerous behavioral and medical benefits for the dog (i.e. a major decrease in aggression, marking, uterine infections, ovarian and mammary cancer, and breeding). With this knowledge being largely emphasized to the public for decades, it has become a very common norm in society to neuter/spay your dog prematurely (prior to their first round of heat) to reap those benefits. Though these precautions are necessary for the dog and the owner, I noticed that there was a lack of research surrounding any possible negative impacts from this procedure.

Thinking back to what Carly had said about the majority of those injured canines being neutered early, I wondered if that was such a coincidence. This led me to phase 1 of my study, where I conducted a data analysis study on the possible association of early neutering/spaying with increased occurrences in osteopathic and neurological disorders in canines. Although my mentor had worked to grant me permission in collecting the de-identified data, unfortunately, due to her job relocation, I was left without a mentor at the start of my data collection as a rising junior. Taking on the first phase of my study without much guidance was intimidating at first, however, throughout this period I learned many crucial lessons within the world of research. One being; to not be discouraged when things don't go according to plan. With or without a mentor, it's certainly possible to educate yourself with the background you need to take on challenges on your own, as long as you have the right resources. However, there were points where I made mistakes or rejected my hypothesis in the end. Combing through data for hours and having some failed attempts with specific statistical tests, only for there to be no significance, discouraged me at times. There were some points where I questioned my research and if I was on the right track with where it was going. With some patience, having some faith in my ideas, and a few all-nighters, I ended up finding some significance.

I was able to find a significant direct relationship between small breed canines having increased development in specific neurological diseases early on in life. Additionally, I found that an increase in neurological diseases in those canines resulted in almost no osteopathic disorders in the future. But the bigger question in my mind: Did early-neutering/spaying have any correlation? In one of the many tests that I ran, I found one test to be significant in partially supporting my hypothesis that early-neutering/spaying did have a correlation to some osteopathic disorders in canines. At this point, questions and ideas kept pouring out of me even with phase 1

being finished. I wanted to dig deeper behind this correlation and find out what was really causing it. This led me to phase 2 for my senior year, being more focused on the hormonal and blood level impacts from an early-neuter/spay procedure. I was fortunate enough at this point to perform my research under the supervision of my recently acquired mentor, Pam Williams, at Norwalk Animal Hospital.

My thoughts were that if I could dig deeper in finding the root of what was causing the development of bone and joint diseases following an early-neuter/spay procedure, I could reveal the source of the correlation. In this case, we could be one step closer to putting up a wall to any significant hormonal changes before they create significant damage. This phase of my study is the project that earned me a spot in the selection for the 2021 Regeneron Science Talent Search.

For phase 2, through a blood sample data analysis study, I analyzed and combed through 8 years of de-identified medical records, analyzing the possible effect of estrogen and testosterone shifts on blood samples from early neutering. Additionally, I took the development of any osteopathic disorders post-CBC (Complete Blood Count) and compared this disease development time period between canines neutered < 6 months and ≥ 6 months. While analyzing past literature, something that particularly stood out to me was that the removal of the gonads (reproductive organs) prevents feedback of estrogen and testosterone on multiple signaling pathways in the canine. Specifically, the hypothalamus, located just on the undersurface of the brain, and the pituitary gland, right below the hypothalamus. The two hormones target all of the major hypothalamic neuroendocrine and autonomic cellular groups to activate multiple signaling pathways in the hypothalamus and pituitary gland. With estrogen and testosterone targeting all of the major pathways to the thyroid gland (T4's source in production), a deficiency of the two would disrupt the role of the hypothalamus in connecting the endocrine system to the nervous system. Overall, this would block its main job; telling the pituitary gland to start or stop making hormones.

With estrogen and testosterone levels decreasing immediately after an early-neuter/spay procedure, this would leave the possibility of early-neutered canines correlating to an underactive thyroid. Consequently, this would impact their blood calcium, phosphorus, and T4 levels. This could lead to low bone mass and microarchitectural deterioration of bone tissue, increasing bone fragility and the risk for developing an osteopathic disease.

Overall, going into a daunting senior year with a global pandemic limiting my options left me with low expectations for phase 2 of my study. After spending months combing through and analyzing data, the moment I hit a breaking point in my research was the best feeling a researcher could have throughout the process. Finding significance wasn't just supporting my hypothesis, but it was about proving to myself that really anything is possible no matter what challenges appear on the journey. It also made me aware of the need for educating the public on this side of early-neutering/spaying that needs more research. After a majority of the patients were neutered/spayed between 1.5-6 months of age, over 50% of them were diagnosed with hypothyroidism. Comparing early-neutered and late-neutered canines, early-neutered canines had developed early signs of patella luxations, cranial cruciate ligament ruptures, or limb lameness within a 5 month period after CBCs were conducted. These results can be applicable to veterinarians, pet owners, and researchers worldwide. Although there is a mission to control overpopulation, it is crucial to realize that this pattern of early neutering has been contributing to these drastic hormonal shifts. This only maximizes the risk for diseases and health issues down the line for the dog. With further research on this topic, I would love to see if the hormonal shifts could significantly alter the phosphate transporters in the gut, bone, or kidney. Looking at the role estrogen and testosterone play in targeting all of the major hypothalamic neuroendocrine signaling pathways to the thyroid gland could reveal the source for decreases in T4 levels.

In making all of these fascinating discoveries, this journey has taught me invaluable life lessons that I will hold onto in college and beyond. I've acquired problem-solving, college-level presentation skills, and the ability to apply mathematics in the science field. Additionally, being able to assess/initiate a majority of this process independently, I've been able to expand my potential as a scientist. All of this has played a huge role in making science, mathematics, and my ideas come to life, and I'm extremely grateful for that. I'm confident that the hard work and skills I've acquired through this journey will help me this fall as I pursue my bachelor's in Pre-Veterinary Medicine at the University of Delaware.

The best advice I could give to any new researcher would be that the possibilities within the world of research are endless. Whether that be a new methodological approach to a study that's already been done, examining a topic from a different angle, or pursuing something that nobody has ever assessed before. I've found that researching a topic that I'm also incredibly passionate about has pushed me farther in expanding my curiosity in the field of animal sciences.

Another important thing to remember is to believe in your ideas and trust your gut during the process. If something is telling you to pursue an idea or try something out you didn't originally plan/hypothesize; do it! One of the most groundbreaking tests I ran in my study wasn't planned and resulted from me noticing specific trends when combing through the data. By trusting my instincts during my analyses, I discovered some of the most important major findings of my research. Even when I questioned my ideas and my capabilities, I still managed to push through and prove that they were possible. Looking back to when I first started this journey, I never imagined how far it could take me. To any new researcher out there; keep going because you never know, you just might surprise yourself in the end.

II. Research Section

Abstract

Dogs have been man's best friend for thousands of years. Unfortunately, their life is commonly cut short due to osteopathic diseases, ranging from mild to severe cases, sometimes even resulting in death. Previous research has found that in some small and large breed dogs, their age of neutering can cause significant hormonal shifts, increasing the number of osteopathic occurrences later in life. There has been a lack of research on these hormonal shifts and what has been truly causing this correlation. Through a blood sample data analysis study, looking at numerous records of early-neutered canines (n=75), I determined a significant direct linear relationship to increased diagnoses in hypothyroidism with early neutering ($p < .0001$). Additionally, I took the development of any osteopathic disorders post-CBC and compared this disease development time period between canines neutered < 6 months and ≥ 6 months. Various breeds of early-neutered/spayed canines had developed early signs of patella luxations, cranial cruciate ligament rupture, or limb lameness in a shorter time period in comparison to canines neutered/spayed ≥ 6 months ($p = .009$). Although an important mission in the veterinary world is to control overpopulation, it is crucial to realize that this pattern of early neutering has been contributing to these drastic hormonal and bone density shifts. This only maximizes the risk for diseases and health issues down the line for the dog.

1.0 Introduction

Everyday, osteopathic diseases affect the lives of thousands of canines. This is evidence that in a broad range of osteopathic issues; the severity can range from mild, degenerative pain, to severe issues, and very often death. Looking at this pattern, it may come as a surprise that a very common surgical procedure performed daily within private veterinary practice and no-kill animal shelters has been having a direct impact on these osteopathic issues. The surgical procedure in question being premature (before one year of birth) neutering and spaying of canines. Dog owners worldwide that have to make the agonizing decision to euthanize their dog due to bone/joint diseases are very often told that the disease was due to "old age", and the dog's genetics alone. Though it's true that certain breeds are naturally prone to developing these diseases (M. Kent, 2018), previous research has found that in some large and small breed dogs, their age of neutering/spaying can correlate to an increase in osteopathic disorders later on in life (A. Hart, 2013, 2016). Digging deeper behind this correlation, looking at the negative impacts that come with neutering/spaying before 12 months of age, drastic shifts in estrogen and testosterone levels accompany the removal of the canine's gonads (S. Rawlinson, 2019). Examining the effects of these hormonal shifts in relation to various breeds of canines' blood Calcium, Phosphorus, and Thyroxine (T4) levels will further portray evidence of the long-term damage that results from early-neutering/spaying.

1.1 Osteopathic Diseases

An "osteopathy" is also called a bone disease. The bone and joint disorders looked at in this study ranged from mild to severe symptoms in various breeds of canines.

1.2 Bone Metabolism

Dog owners and veterinarians worldwide expect canines to end up with an osteopathic disease at some point in their life because of their hereditary history and because this is how canines have ended up for years. The growth of the skeleton, its response to mechanical forces, and its role as a mineral storehouse; are all dependent on the proper functioning of circulating hormones that respond to changes in blood calcium and phosphorus. If calcium or phosphorus are in short supply, the regulating hormones take them out of the bone to serve vital functions in other systems of the body. Too many withdrawals can weaken the bone. This loss of bone mass leads to the development of structural abnormalities that make the skeleton more fragile, leading

to osteoporosis, making the dog's risk for developing an osteopathic disease drastically higher (L. Sanburn, 2007).

1.3 Neutering and Spaying

Despite the genetic component that contributes to the development of these debilitating diseases, societal contributing factors may play a role in this as well. One of these factors can include early-neutering and spaying (Kent, M. 2018). Many people across the country neuter and spay their dogs before one year of age for a multitude of reasons. There are benefits that include a major decrease in aggression, marking, uterine infections, ovarian and mammary cancer, and breeding (F. Pollari, 1996). Though these preventions are beneficial for the owner and the dog short-term, there may be various risks that could affect the dog long-term. Removing the reproductive organs can drastically decrease certain hormones such as estrogen, progesterone, testosterone, and androgen (B. Bonett, 2003). These hormones coordinate everything from digestion and growth in appetite, as well as immune function (X. Yang, 2017). Hence, the significant amount of weight gained shortly after the early-neutering/spaying. Neutering reduces marking in 80% of dogs with a marked improvement in 40% (Horwitz, D. 2012). This view of early-neutering has evidently not been shared to different parts of the world, resulting in a lack of knowledge on this topic.

1.4 Contrasting Views

This research has caused a great deal of controversy over the years. Neutering and spaying is commonly avoided and not generally promoted by animal health authorities in many European countries. These views are contrasted to the U.S. in various ways, one being: only 20% of dogs are intact (Swartz, A. 2015). Previous studies have shown that 99% of dogs were gonadally intact in Sweden, there was a 57% intact rate reported in Hungary and a 46% intact rate reported in the United Kingdom (Swartz, A. 2015). The main reason for these health authorities not recommending early-neutering/spaying is because of the many risks that accompany it. (Bentley, A. 2018, Hart, A. 2013, 2016).

1.5 Negative Effects

One of the risks that can result from early-neutering/spaying is a drastic change in hormone levels. After dogs are neutered/spayed prematurely, their testosterone, estrogen, calcium, and phosphorus levels may decrease dramatically (J. Shaker, 2018). Thus, neutering/spaying after 1 year of age or after their first round of heat may prevent this from

happening (Bentley, A. 2018). These hormonal decreases then lead to a major decrease in bone mineral density. Low bone mineral density levels signify a diagnosis of osteoporosis, which in turn, leads to increased occurrences in joint diseases and fractures (J. Xia, 2019).

1.6 Connections to Human Physiology

Since canines and humans share some common anatomy and physiology, they may react to early-neutering/spaying in a similar fashion. For example, women who have completed menopause are more prone to arthritis, and men are twice as likely to develop prostate cancer after undergoing a vasectomy (Lisabeth, 2012). This is due to the major effect on the gonadal hormones, estrogen, testosterone, and progesterone. This can relate to the controversial issue regarding early-neutering/spaying as both species' hormone levels are being shifted. As this also applies to human anatomy in a similar fashion, the importance of knowing these risks and acting upon them early can not only benefit dog owners but society as a whole.

2. Purposes

A current issue that exists today is the possible association between lowered blood calcium, phosphorus, T4 levels in early-neutered canines. The effect of early-neutering/spaying on these hormones in conjunction with the development of osteopathic disorders has lacked research in previous years. My goal was to determine significant differences between these calcium, phosphorus, and T4 levels in early-neutered and late-neutered/intact canines. A possible correlation between these possible contributing factors amongst the development of osteopathic disorders may justify why this correlation exists.

3. Hypotheses

Hypothesis 1: Selected canines that were neutered/spayed before 1 year of age will have lower levels of T4 in the blood.

Hypothesis 2: Selected canines that were neutered/spayed before 1 year of age will have lower levels of blood calcium.

Hypothesis 3: Selected canines that were neutered/spayed before 1 year of age will have lowered levels of serum phosphorus.

Since we can presume that the early-neutered canines' estrogen and testosterone levels faced drastic decreases (L. Sanburn, 2007), there may have been prevented feedback on the production of various hormones in the thyroid gland and parathyroid gland. This, in turn, would leave the canine with underactive hormone production, leading to significant changes in the canines' bone

mineral density (I. Lieberman, 2020). Over time, these contributing factors may play a significant role in the development of osteopathic issues later on in life.

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4.0 Methodology

In an attempt to ascertain the hypothesized significance between early-neutered canines and differences in blood calcium, phosphorus, and T4 levels, along with increased occurrences in osteopathic diseases, de-identified blood samples and medical history for all selected canines from 2012-2020 were obtained from Norwalk Animal Hospital, Norwalk, CT.

4.1 Student vs. Mentor Role

In this study, my mentor provided me with all de-identified medical records from 2012-2020, including all chemistry panels with complete blood counts (CBC) for all canines (n=75). I combed through, classified, and analyzed all of the data on my own.

4.2 Selected Canine Qualifications

This study required selected canines to have accessible lab records following the parameters of only looking at all chemistry panels with CBCs and a total T4 count. Any medical history signifying the canine having an osteopathic disorder, pre or post-CBC, was also taken into account. If patients with an osteopathic disease did not show any sign of low blood sample levels, these patients were further evaluated in determining a probable cause for this, including if the breed was playing a role in the development of these diseases. Certain breeds such as the Golden Retriever, German Shepherd, Labrador Retriever, Great Dane, Saint Bernard, Dachshund, Chihuahua, and the miniature poodle are most commonly affected by orthopedic conditions such as patellar luxations, osteoarthritis, and hip dysplasia. Being naturally prone to developing these diseases could have contributed to the possibility of the owner providing Vitamin D or Calcium supplements in the canine's everyday diet or medication. In this case, hormonal levels could have been altered, resulting in the possibility that some of the canines with osteopathic diseases had normal blood calcium, phosphorus, or T4 levels in the lab tests.

4.3 Data Acquisition

First, I looked into the Antech Diagnostics and Imaging medical records to find canine patients labeled with a description of chemistry panels with CBCs. I then looked that patient up in the hospital's lab records and recorded normal T4, calcium, and phosphorus ranges. Actual levels of that patient were then recorded. Any blood sample level that fell below the normal

range signified a case of hypocalcemia, hypophosphatemia, or hypothyroidism. In order to obtain certain patients that had records of a total T4 count, a code was designed in order to select certain canines with those specific lab results.

4.4 Coding

At this point in the data collection, another member of the science research program was consulted to help code a program to aid in data analysis. Canines with the labels of "Chem panel", "CBC", and "T4" needed to be identified within 3 records. Each record contained around 850 patients so it would be inefficient to find all dogs fitting this description by hand. The code, written in Java, ran a linear search algorithm to output all the names of dogs fitting this description, their patient number, and owner number.

Lastly, I looked into each of these patients' record history to see if they had developed any osteopathic diseases before or after they had their CBC. This would help in understanding if the canine possibly had low hormone levels that contributed to disease development before it was detected.

4.5 Classifications

These patients were de-identified and classified into their gender, breed, age when neutered/spayed or if they were intact, CBC date, phosphorus, calcium, and T4 levels, osteopathic issues, and when those osteopathic issues were first diagnosed/noticed. These classifications were put into an Excel spreadsheet.

4.6 Limitations

Certain patients without T4 levels did not qualify for a complete data set. If they had been, variances would have been different between canines with and without T4 levels. Maintaining these constant variables were crucial in finding accurate significance.

Being that various breeds were examined, specific breeds may have been genetically more sensitive to the hormonal changes that resulted from an early neuter/spay procedure. Their growth plates may have also closed at a different rate than other breeds. Additionally, there are other contributing factors to blood calcium, phosphorus, and T4 levels, such as diet, dehydration, and genetics. However, the selected breeds of canines had normal calcium, phosphorus, and T4 levels when the procedure took place.

4.7 Data Analysis

This was strictly a data analysis study with no observation of the actual rehabilitation for the disease itself, as it did not affect the outcome of this analysis. Numerous linear regressions, correlation tests, and a bar graph were constructed in the hope of finding significant hormonal differences between early and late-neutered/spayed canines. The p value was set at < .05.

5.0 Results

Table 1. Linear Regression Relationships Between Canines' Age of Neutering/Spaying with Phosphorus, Calcium, and T4 Levels in the Blood (n=73) * indicates significance $p < .05$.

	Coefficients	*Standard Error*	*t Stat*	***P-value***	*Lower 95%*	*Upper 95%*	*Lower 95%*	*Upper 95%*
Intercept								
Phosphorus	0.05	0.03	2.76	0.1	-0.03	0.43	-0.03	0.43
Calcium	0.03	0.02	2.97	0.09	-0.03	0.42	-0.03	0.42
T4	0.07	0.02	16.53	**0.0001***	0.25	0.65	0.25	0.65

Table 1 shows a significant linear regression of early-neutered canines to lowered T4 levels (p = .0001); early-neutering did not show a significant correlation to the canines' blood calcium (p = .09) or phosphorus levels (p = .10). Significance in T4 levels were further demonstrated in a linear regression graph below.

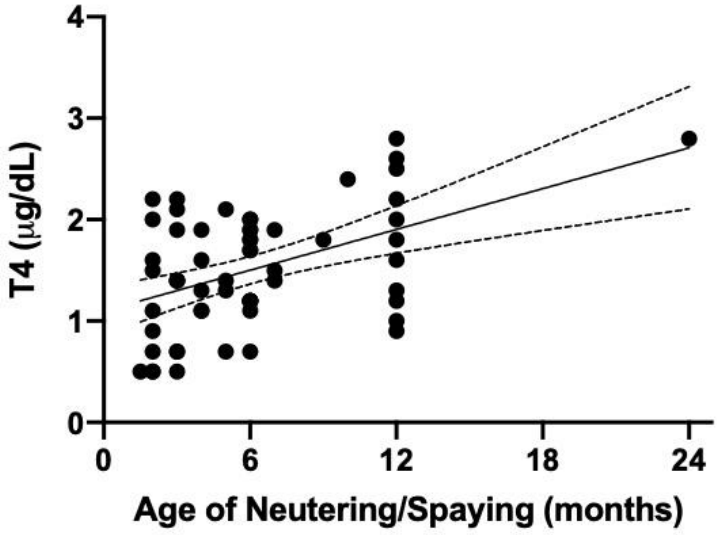

Figure 1. Significant Linear Relationship Between Canines' Age of Neutering/Spaying with T4 Levels in the Blood (n=62) A significant trend was found. ($r^2 = .24$) (p = .0001)

Linear Regression showed a significant direct relationship between various breeds of early-neutered canines that had a total T4 count, excluding 11 of the patients. Over 50% of early-neutered canines (1-6 months) fell below the 1µg/dL mark in comparison to intact/late-neutered breeds. Any T4 levels lower than 1µg/dL supported the diagnosis in hypothyroidism.

Table 2. Linear Regression Between Canines' Age of Neutering/Spaying and Blood Calcium (n=73)

	Coefficients	*Standard Error*	*t Stat*	***P-value***	*Lower 95%*	*Upper 95%*	*Lower 95%*	*Upper 95%*
Intercept	9.88	.13		.001	9.61	10.14	9.61	10.14
Calcium	0.03	0.02	2.97	0.09	-0.03	0.42	-0.03	0.42

Table 2 attempted to perceive a significant association between early-neutered canines and low blood calcium levels. This linear regression showed no correlation between the two variables (p = .09). This insignificance signifies that the earlier the canines were neutered/spayed, no impact was made on his/her blood calcium levels in the future.

Table 3. Linear Regression Between Canines' Age of Neutering/Spaying and Serum Phosphorus (n=73)

	Coefficients	*Standard Error*	*t Stat*	***P-value***	*Lower 95%*	*Upper 95%*	*Lower 95%*	*Upper 95%*
Intercept	3.74	0.22		.0001	3.30	4.20	3.30	4.20
Phosphorus	0.05	0.03	2.76	0.1	-0.03	0.43	-0.03	0.43

Table 3 shows the insignificant linear regression between early-neutered canines and low serum phosphorus levels (p = .10). This relationship did not have a significant correlation, signifying that the earlier the canines were neutered/spayed, the occurrence of hypophosphatemia did not increase.

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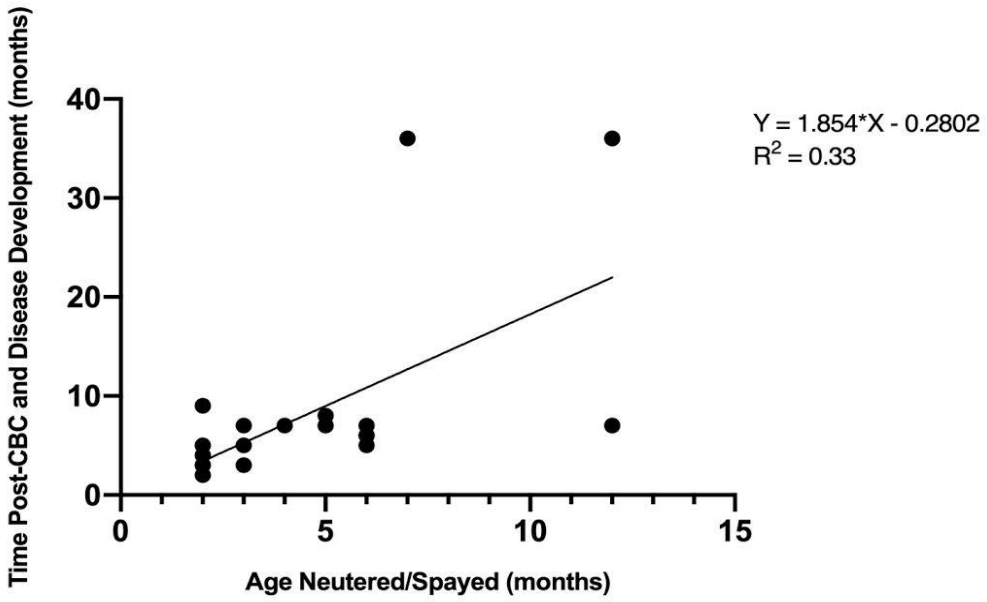

Figure 2. Significant Linear Regression Demonstrating Osteopathic Disease Development Time Period Post-Blood Sample Collection in Association to Canines' Age of Neutering/Spaying (n=19)
A significant trend was found. ($r^2 = .33$) ($p = .009$)

As shown in Figure 2, various breeds of early-neutered/spayed canines that had developed a patella luxation, cranial cruciate ligament rupture, or limb lameness were regressed on the time period it took for them to develop principal signs of these disorders post-blood sample collection. A significant trend was found between the two variables ($p = .009$). Using the patients that had developed an osteopathic disease (n=19), the earlier they were neutered/spayed, they developed 1 out of 3 of these diseases within a 5-12 month period. The mean disease development time periods for the early and late-neutered/spayed canines are shown below.

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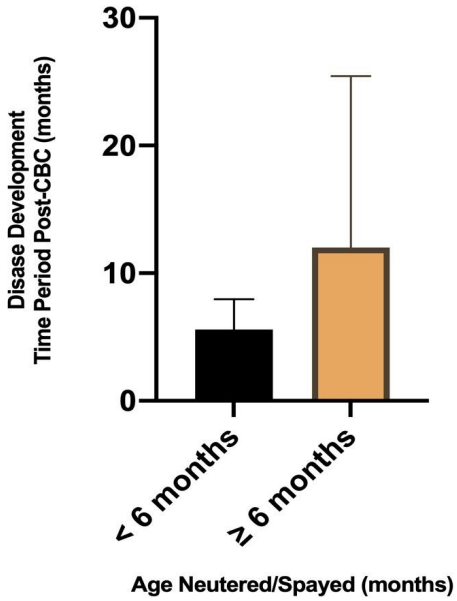

Figure 3. Mean and SD Bar Graph for Early and Late-Neutered/Spayed Canines With Osteopathic Diseases and Their Disease Development Time Period Post-CBC (n=15)

With a total of 19 canines that developed an osteopathic disease, 15 out of those 19 had either developed a patellar luxation, a rupture in their cranial cruciate ligament, or limb lameness. The time period it took for the development of the first symptoms of these diseases was compared between 5 late-neutered and 10 early-neutered canines. The average time it took for the early-neutered canines to develop symptoms of these disorders was 5.6 months, while the average time it took for late-neutered canines to develop these symptoms was 12 months post-CBC.

6.0 Discussion

Through a linear regression conducted on 73 canines, a significant correlation amongst early-neutered canines (<12 mo) with decreased levels of T4 in the blood was found (See Table 1) ($p = .0001$). Differences in blood calcium and phosphorus levels did not show any significance to the canines' age when neutered/spayed ($p = .09$) ($p = .10$).

Hypothesis 1. T4 levels in the canines' blood appeared to have a significant direct linear relationship to the age they were neutered/spayed ($p = .0001$) ($r^2 = .24$) (See Figure 1). This

supports my first hypothesis, and rejects the null, being that the earlier the canine was neutered, the lower the T4 levels were later on in life. As shown in this graph, each canine that had a T4 level below 1, signified a case of hypothyroidism. Evidently, the majority of these canines with low T4 levels had very similar neuter/spay ages. Over 50% of these canines had been neutered/spayed between 1.5-6 months of age. As these canines were neutered/spayed prematurely, it can be presumed that drastic hormonal decreases in their estrogen or testosterone resulted from this gonadal removal (A. Hart, 2013). A probable cause for this significant correlation could be due to the removal of the gonads preventing feedback of estrogen and testosterone on the pituitary gland and hypothalamus. The role of the hypothalamus is to connect the endocrine system with the nervous system. Its main job; to signal the pituitary gland to stop or start producing various hormones (J. Biran, 2015). The thyroid-stimulating hormone (TSH), is produced in the anterior pituitary gland and regulates endocrine function in the thyroid gland. Ultimately, estrogen and testosterone target all of the major hypothalamic neuroendocrine and autonomic cellular groups to activate multiple signaling pathways to the thyroid gland, T4's source in production (L. Sanburn, 2007). Since these early-neutered canines' estrogen and testosterone levels faced drastic changes, there was prevention in feedback on the production of various hormones in the thyroid gland. This, in turn, would leave the canine with an underactive thyroid (I. Lieberman, 2020). If this goes undetected – as some canines do not get an annual CBC – this could result in osteoporosis in the future. Thyroid hormones are essential for skeletal development and bone homeostasis (R. Williams, 2018), so it is vital that these hormones get checked at least once a year to prevent this disease development early on in life.

Hypothesis 2. Blood calcium levels in various breeds of canines did not demonstrate a significant correlation to the age at when they were neutered/spayed ($p = .09$) (See Table 2). This did not support my second hypothesis, which supported the null, being that the earlier the canines were neutered/spayed, there were no significant trends to low levels of calcium or hypocalcemia being present in the blood. Blood calcium is regulated by the parathyroid gland (PTH). Whenever blood calcium levels may be low, the PTH increases blood calcium levels by stimulating osteoclasts, which in turn, would end up breaking down bone to release calcium into the bloodstream (W. Grünberg, 2014). It was hypothesized that early-neutered/spayed canines would have a significant correlation to hypocalcemia due to the role estrogen and testosterone play in calcium absorption (J. Gallagher, 1980). In addition, making comparisons to humans, a

previous study found that estrogen therapy improved calcium balance in patients with postmenopausal osteoporosis (J. Gallagher, 1980). Since canines and humans share some common anatomy and physiology, they may react to early-neutering/spaying in a similar fashion. As women after completing menopause face drastic decreases in estrogen, canines also face dramatic changes in their estrogen and testosterone immediately following their premature neuter/spay procedure (A. Hart, 2016). The reasoning behind this insignificant correlation could be due to falsely increased blood calcium levels by diet and dehydration (M. Peterson, 2018). Further research into this correlation by taking the blood protein, albumin, into account would confirm that the canine is not dehydrated. Additionally, taking the canine's diet into consideration and examining if any calcium supplements were included, would aid in the understanding of the calcium levels presented in the CBC blood sample.

Hypothesis 3. Serum phosphorus levels in various breeds of canines did not show a significant correlation to the age they were neutered/spayed ($p = .10$) (See Table 3), ultimately not supporting my hypothesis, but supported the null. The earlier these canines were neutered/spayed, there seemed to be no trend in having increased occurrences in hypophosphatemia. The presence of adequate amounts of phosphate is critical for the process of apoptosis of mature chondrocytes in the growth plate (J. Meng, 2010). Without the presence of serum phosphorus in high enough quantities, the generation of new bone will be blocked, resulting in delayed growth and osteoporosis (M. Penido, 2012). Phosphate transporters in the gut, bone, and kidney are key in regulating phosphate homeostasis (J. Marks, 2009). It was hypothesized that the serum phosphorus levels would be directly affected by the drastic hormonal shift that results from early-neutering/spaying. If this is possibly still the case, it is possible that this hormonal shift alters the phosphate transporters in the gut, bone, or kidney. Looking into this possible correlation could further demonstrate if there is still a significant correlation, despite the insignificance demonstrated in Table 3.

Figures 2 and 3. Although the correlations presented in these graphs were not originally hypothesized, these tests were conducted after noticing specific trends amongst the canines neutered/spayed at < 6 months of age, in comparison to the canines neutered/spayed ≥ 6 months. Shown in Figure 2, various breeds of canines neutered/spayed < 6 months that had developed a patella luxation, cranial cruciate ligament rupture, or limb lameness were regressed on the time period it took to develop principal signs of these disorders, in comparison to canines

neutered/spayed ≥ 6 months. The disease development time period was classified as the amount of time post-CBC/diagnosis of low blood sample levels, up until the date that their first symptoms were reported. Early symptoms that were taken into account included the canine's inability to jump, lack of mobility, and stiffness in the stifles. As shown in the graph, a significant trend was found between the two variables ($p = .009$). Overall, this showed that using the patients that had developed an osteopathic disease (n=19), the earlier they were neutered/spayed, they developed 1 out of 3 of these diseases within a 5-12 month period. (See Figure 3) The average amount of time for the disease development in canines neutered/spayed < 6 months was 5.6 months to develop early symptoms. While the average amount of time for the disease development in canines neutered/spayed ≥ 6 months of age took 12 months to develop principal signs of joint disease.

6.1 Applications

The acceptance of these trends would benefit the veterinary field, pet owners, and researchers worldwide. For veterinarians, various breeds of early-neutered canines developing swelling/stiffness in their stifles and limb lameness in a significantly reduced amount of time may help to improve the timeliness of disease diagnosis. Additionally, although there is a mission to control overpopulation, it is crucial to realize that this pattern of early neutering has been contributing to these drastic hormonal shifts. This only maximizes the risk for diseases and health issues down the line for the dog. Not only could this educate the pet owners and veterinarians of the world, but due to the similar physiological and anatomical level between canines and humans, there are many similar issues that could have an effect on society, and the world as a whole. Women who have completed menopause are more prone to osteoarthritis due to their estrogen levels decreasing dramatically, along with ovarian functions declining with age (Ji, M. 2015). Men are also two times more likely to develop prostate cancer after undergoing a vasectomy, decreasing their testosterone hormone levels dramatically (Huang, X. 1995). Neutering/spaying dogs at a premature age is such a controversial issue due to the short-term benefits, that this could potentially spark a debate at weighing short vs. long-term benefits. The question that remains; do the short-term behavioral benefits that result from premature neutering/spaying outweigh the long-term health implications down the line for the dog? Pet owners and animal rescues should consider this. Knowing when the major growth plates in your dog close, or waiting until his/her first round of heat is over, will allow the growth plates to

gradually thin as hormonal changes approaching puberty signal the growth plates to close on their own. At this point, the growth plates will become a stable and inactive part of the bone, leaving them less vulnerable to injury (A. Bentley, 2018).

6.2 Future Research

Looking at various breeds of canines, if there could be a solution that would allow any canine to be neutered early, while preserving both the short-term benefits that accompany it, and most importantly, preserves the canine's bone mineral density, then this pattern of osteopathic disease development early on in life has the potential to be broken. A possible medicine/supplement for the dog to take prior to their procedure that would preserve those hormonal levels, or that would keep those levels balanced could make the risk of developing osteoporosis significantly lower. In this case, the growth plates would not prematurely close. This could also delay the onset of certain osteopathic diseases, or overall make them less severe. Additionally, it's possible that the hormonal shifts could significantly alter the phosphate transporters in the gut, bone, or kidney. Looking at the role estrogen and testosterone play in targeting all of the major hypothalamic neuroendocrine and autonomic cellular groups to activate multiple signaling pathways to the thyroid gland could reveal the source for these decreases in T4 levels. With further research on these topics, we will be one step closer to putting up a wall to these significant hormonal changes before they create significant damage.

7.0 Conclusions

In this study, we sought to gain insight on the effect of early-neutering/spaying on T4, calcium, and phosphorus levels in the blood, in various breeds of canines. I analyzed and combed through 8 years of de-identified medical records, analyzing the possible effect significant hormonal shifts would have on blood samples from early neutering. Additionally, I took the development of any osteopathic disorders post-CBC and compared this disease development period between canines neutered < 6 months and ≥ 6 months. A significant direct linear relationship was found between canines that were neutered early with increased occurrences in hypothyroidism. Another significant trend was found through the use of the patients that had developed an osteopathic disease. The earlier they were neutered/spayed (1.5-6 months), they had developed a patellar luxation, a rupture in the cranial cruciate ligament, or limb lameness within a 5-12 month period after CBCs were conducted. Knowing the risks associated with

neutering/spaying your dog before they reach sexual maturity and the natural risks associated with your breed's size is vital in protecting the dog's life from being cut short from a debilitating disease. This will make both the dog owner and canine community life more enjoyable with the hope of your dog living a longer life.

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