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Stem Cell Therapy for Canine and Feline Patients with Chronic Kidney Disease

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Murray State University Honors College

HONORS THESIS

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Stem Cell Therapy for Canine and Feline Patients with Chronic Kidney Disease

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Abstract

This research aimed to statistically analyze the relationship between stem cell therapy and chronic renal failure in a sample of canine and feline patients. Stem cell therapy is a burgeoning field in both human and veterinary medicine, seeking mainly to better understand disease incidence and repair damaged tissues, such as those involved in spinal cord injuries and Alzheimer's disease. In relation to veterinary medicine, prior research suggests that stem cell therapy has potential to be an effective tool against diseases such as acute kidney injury and chronic kidney disease. This project examined how autologous adipose-derived mesenchymal stem cells affected the rehabilitation of the patient as an adjunctive treatment to fluid diuresis and drug therapy. This was done primarily by examining comprehensive chemistry panels and kidney health profiles taken during treatment of the patients and making statistical inferences from the data set using single factor analysis of variance models. These values were then compared to healthy patient reference intervals as a measure of efficacy.

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Literature Review

Introduction

Chronic kidney disease (CKD) is the most common small animal kidney disease. It tends to affect older animals, as in canines, but can be diagnosed at any age. Approximately 7% of dogs are diagnosed with this disease. During diagnosis, chronic kidney disease must be differentiated from acute kidney injury (AKI), as treatment and prognosis are affected significantly by that distinction. Chronic kidney disease is defined as the disease being present three or more months with progressive loss of function, whereas “acute kidney injury is defined by sudden damage to the renal parenchyma and may be accompanied by an abrupt decrease in glomerular filtration rate (GFR)” (Dunaevich, 2020, p. 2508). Polzin (2011) states that distinctions between the two can be found by observing body condition score, duration of loss or suppression of appetite, hair coat condition, urine volume changes, kidney size, bone strength, as well as other observable clinical signs. Chronic kidney disease is often characterized by weight loss, appetite loss and polyuria/polydipsia for three months or longer, small kidneys as compared to expected organ size for the patient, weakened bones, and anemia not due to hemorrhage. Dunaevich (2020) notes that the most commonly reported clinical signs are anorexia, lethargy, and vomiting. This disease is considered irreversible as well as progressive, while acute kidney injury has potential to be reversible. Acute kidney injury has an approximately 50% mortality rate. Furthermore, patients can develop acute on chronic kidney disease where both conditions are present concurrently, as seen with a stable CKD patient sustaining an acute reduction in renal function.

Diagnostics

For clinical diagnosis, blood and urine tests need to support the findings of the physical examination. The International Renal Interest Society (IRIS) compares blood pressure, kidney function, and urine protein levels to place the disease into one of four stages. As such, this staging allows for specific treatments respective to the progression of the disease. “Glomerular filtration rate (GFR) has long been considered the golden standard. However, a direct measure of GFR is a time-consuming and labor-intensive process” (Kim, 2020, p. 1130). Due to those factors GFR is often bypassed in favor of other common clinical blood markers including elevated blood urea nitrogen concentration and serum creatinine concentration, hyperphosphatemia, hyper- or hypo-kalemia, hypoalbuminemia, and metabolic acidosis. Urine markers include poorly concentrated urine, proteinuria, cylindruria, renal hematuria, inappropriate urine pH level, inappropriate glucosuria, and mineralization. The International Renal Interest Society has set guidelines for each stage with respect to serum creatinine, as well as arterial stages for both canines and felines. Serum creatinine (in mg/dL) values in canines are; less than 1.4 in stage one, 1.4-2.0 in stage two, 2.1-5.0 in stage three, and greater than 5.0 in stage four. Felines are shifted slightly to less than 1.6 in stage one, 1.6-2.8 in stage two, 2.9-5.0 in stage three, and greater than 5.0 in stage four. Arterial stages start at stage zero and peak at stage three. Canines and felines share values for systolic and diastolic blood pressure at each stage. In mmHg, systolic blood pressure is less than 150 at stage zero, 150-159 in stage one, 160-179 in stage two, and greater than or equal to 180 in stage three. In the same manner diastolic pressure is staged as less than 95 in stage zero, 95-99 in one, 100-119 in two, and greater than or equal to

120 in three. In addition, the American College of Veterinary Internal Medicine classifies proteinuria (as a ratio to creatinine) for canines as proteinuric at values greater than 0.5, borderline proteinuric between 0.2 and 0.5, and non-proteinuric at less than 0.2. Felines are classified as proteinuric at ratios greater than 0.4, borderline proteinuric at 0.2-0.4, or non-proteinuric at less than 0.2. “The stage of CKD is based on the level of kidney function as measured by the patient’s serum creatinine concentration” (Polzin, 2011, p. 17). Ideally patients are staged from two or more fasted samples while maintaining proper hydration, and tracked over multiple weeks to observe the stability of the disease. To better define the stages, multiple samples of urine should be taken to confirm proteinuria and blood pressure should be measured a few times over two or more weeks.

Other biomarkers for chronic kidney disease in canines have been recognized more recently by the International Renal Interest Society. Symmetric dimethylarginine (SDMA) has exhibited the ability to diagnose chronic kidney disease as early as stage two, while cystatin C (CysC) has shown potential to diagnose as early as in stage one. Kim’s (2020) study notes that serum SDMA is not affected by as many non-renal factors as creatinine. As such, it has exhibited the ability to diagnose chronic kidney disease roughly 10 months earlier in canines and 17 months earlier in felines when compared to observing elevated creatinine levels. “CysC is a cysteine protease inhibitor that is filtered by the glomerulus and unaffected by non-renal factors such as inflammation, age, and sex” (Kim, 2020, p. 1130). Kim (2020) concludes that both healthy canines and those with non-kidney diseases exhibit much lower concentrations of cystatin C than those in various stages of chronic kidney disease. He also acknowledges that

concentration of cystatin C is strongly related to concentrations of symmetric dimethylarginine and creatinine. Additionally, “CysC was the most effective biomarker for detecting early stage CKD (IRIS stage I); and CysC was a more sensitive marker of early or late stage CKD than CREA or SDMA” (Kim, 2020, p. 1136).

Treatment

Currently, aside from dialysis and transplantation, clinical studies appear to suggest that changing the diets of canines in stages three or four and felines in stages two through four to a diet formulated to assist kidney health is the factor that most increases long-term outlook. When compared to maintenance diets, renal diets are often found to bolster not only the nutrition but also the quality of life of the patients with renal disease. Renal diets are specially formulated to include “reduced protein, phosphorus, and sodium content, increased B-vitamin and soluble fiber content, increased caloric density, neutral effect on acid-base balance, supplementation of omega-3 polyunsaturated fatty acids, and addition of antioxidants” (Polzin, 2011, p. 18). Feline renal diets also contain supplemented potassium. Effectiveness of the change in diet can be measured through body condition score, body weight, appetite, packed cell volume, and serum albumin concentration. Other studies have suggested that renal diets can also exhibit positive effects on blood urea nitrogen and creatinine concentrations.

Dunaevich (2020) acknowledges that chronic kidney disease is frequently diagnosed in later stages “when renal functional impairment exceeds compensatory mechanisms, and irreversible, severe renal parenchymal damage already has occurred” (p. 2508). This is attributed to current technology lacking the ability to recognize

markers in earlier stages. Due to this lack of early diagnosis through blood urea nitrogen and creatinine, stem cell therapy has been seen as a potential method of preventing further damage. However, as noted in the diagnostics section, symmetric dimethylarginine and cystatin C are able to help identify chronic kidney disease earlier than the biomarkers used at the time the data for this study was recorded. Human medical studies utilizing stem cell treatments hope to eventually be able to regenerate damaged tissues that otherwise would not naturally. This would have important implications for the veterinary medical field as well, allowing patients with chronic kidney disease to have their disease treated instead of managed. “Stem cells have promising potential as a form of regenerative medicine for kidney diseases due to their unlimited replication and their ability to differentiate into kidney cells *in vitro*” (Wong, 2021, p. 914).

Stem Cells

Stem cells are cells in the body that can self-renew and differentiate into multiple types of cells. There are multiple categories of stem cells that are potentially useful. Each of the following types are defined by Wong (2021). Embryonic stem cells (ESCs) are pluripotent cells of the embryo, meaning they are able to differentiate into any cell in the body when induced to do so. Induced pluripotent stem cells (iPSCs) are adult fibroblasts that are changed into a cell analogous to ESCs, using specific transcription factors to achieve this desired cell type. Endothelial progenitor cells (EPCs) play an important part in repairing endothelial damage, as well as maintaining integrity of the vasculature. “EPCs can be isolated from different cell sources, mainly from the readily available bone marrow, cord blood, and peripheral blood” (Wong, 2021, p. 917). Mesenchymal stromal cells (MSCs), including bone marrow MSCs (BM-MSCs), are

readily available in peripheral blood, the umbilical cord (UC-MSCs), adipose tissue (AD-MSCs), skeletal muscle, and multiple other locations in the body.

Embryonic stem cells are considered to have the highest clinical potential. Multiple research groups have created tests with mouse ESCs to show their ability to interact with the kidney's compartments, as well as inducing them to differentiate into specific cells and integrate them into the desired tissue. However, despite the great potential, ESCs are not free of problems. Currently there is legal and ethical opposition to the use of ESCs that slow studies seeking to use them for new therapies. Furthermore, they are particularly tumorigenic compared to the other types of stem cells available, compounded by their allergenicity due to expression of surface proteins.

Induced pluripotent stem cells are regarded as a historic discovery in the field. They are derived from adults to address the ethical concerns, which also means they retain some genetic "memory" of the parent cell so as to not trigger the immune system response. Furthermore, this also suggests that using the homologous cells to create the iPSCs is preferable, as this memory can hinder differentiation if derived from non-kidney cells. iPSCs can be derived from renal tubular and epithelial cells. In turn, this allows researchers to "differentiate [human iPSCs] into kidney lineages or three-dimensional structures of the kidney" as well as establish a "multistep differentiation protocol to induce human iPSCs to differentiate into renal progenitors capable of constructing three-dimensional proximal renal tubule-like structures *in vitro*" (Wong, 2021, p. 917). Additionally, these stem cells have been used in an animal trial to improve an acute kidney injury. Multiple research groups have created kidney organoids *in vitro* that mimic the human kidney and its diverse cell types found throughout.

Endothelial progenitor cells (EPCs) are readily available and crucial to repairing endothelial damage. Studies done with both chronic and acute kidney disease have shown potential viability. In “an animal model of renal ischemia/reperfusion (I/R)-induced AKI, renal artery-derived EPC-like cells integrated into the endothelium after AKI, led to decreased levels of serum creatinine (SCr) and albuminuria while blood flow improved” (Wong, 2021, p. 918). Similarly, in a case with CKD, EPCs derived from bone marrow stopped inflammation from hindering kidney function while simultaneously maintaining function and structure.

Mesenchymal stromal cells (MSCs) have varying effects, seemingly dependent on where they are derived from. Bone marrow MSCs (BM-MSCs) are the most common clinical treatment source of MSCs, but lose much of their ability to replicate and differentiate as the donor ages. This discovery eventually led to the use of different sources for isolating MSCs. Multiple studies suggest that MSCs are “among the most efficient type of cell population for activating regeneration in a damaged kidney” (Wong, 2021, p. 918). Many articles have expressed evidence suggesting MSC effectiveness in experimental chronic kidney disease treatment as well as demonstrating their use in preventing an unrecovered AKI progressing into CKD. Regardless of route of administration, regeneration was observed in patients, citing the MSCs’ ability to differentiate into renal cells. These MSCs then divide into either general kidney cells or into component specific cells.

Umbilical cord-derived mesenchymal stromal cells (UC-MSCs) give new life to medical waste, allowing for isolation of MSCs with low immunogenicity, higher proliferation capacity than bone marrow and adipose tissue MSCs, and retention of cell

potency for many generations. Research continues on UC-MSCs to study the extent of their efficacy, but the studies done previously have shown great promise in their protection of kidney cells, reduced inflammation, and other beneficial factors not found in other MSC derivatives.

Adipose tissue-derived mesenchymal stromal cells (AD-MSCs) are readily available and can be harvested in a minimally invasive manner. Recently they have shown promise as a prominent location for harvesting MSCs due to the relative ease at which they are available, especially as compared to bone marrow MSCs. AD-MSCs have demonstrated usefulness in acute kidney injury therapy, but have been relatively less effective at proliferation and regeneration than their bone marrow derived counterparts.

Preparation of stem cell therapy varies slightly in different studies, but a general method could be as follows. “Adipose-derived MSCs were isolated from adipose tissue … from a subcutaneous site on the ventral abdomen just caudal to the umbilicus …” (Quimby, 2016, p. 166). These cells can then be used immediately or cryopreserved in liquid nitrogen for up to a year before use. The adipose tissue is then washed twice with a phosphate buffered saline and prepared for culture. Quimby’s (2016) study found that:

> For isolation of the stromal vascular fraction, the tissue was minced and digested with 1 mg/ml collagenase (Sigma-Aldrich) for 30 mins at 37°C. The sample was centrifuged, and the stromal vascular fraction was plated in MSC medium (low-glucose Dulbecco’s Modified Eagle Medium [DMEM], 100 U/ml penicillin, 100 µg/ml streptomycin, 2 µM L-glutamine, 1% essential amino acids without

L-glutamine, 1% non-essential amino acids, 0.075% sodium bicarbonate [Invitrogen/Gibco] plus 15% FBS [Cell Generation]). (p.166-167)

After this process the stem cells are incubated until 70% confluence. The growth media must be changed every 2-3 days. “Cells were harvested by trypsinization at passage 2–3, washed and resuspended in 10 ml [phosphate buffered saline] with 200 IU heparin sulfate, and administered as a slow IV push to the cephalic vein over 20 mins” (Quimby, 2016, p. 167). For the particular study referenced the patients received treatments at two week intervals, but this is not necessarily the case in clinical practice.

Statistical Analysis

To compare the values between the different patients, an ANOVA, or analysis of variance, analytical method will be employed. This is used alternatively to repeating a sequential t-test. Analysis of variance is used to attempt to resolve the issue that “even though the null hypothesis is true, the probability of rejecting it increases, whereby the probability of concluding that the alternative hypothesis (research hypothesis) has significance increases, despite the fact that it has no significance” (Kim, 2017, p.22). The ANOVAs are followed by a post-hoc t-test if found to be significant. The significance level observed is variable by the number of divisions made, among other distinctions.

Background

Blood urea nitrogen (BUN), creatinine (CRE/CREA), and phosphorus (PHOS) will be used in the analysis of variance (ANOVA) tests as the primary outcomes of interest. These were chosen over cystatin C (CysC) and symmetric dimethylarginine (SDMA) because CysC and SDMA were not recognized as renal function indicators at the time

of treatment. Each of the three panel values are generally indicative of the kidney's filtration ability. Blood urea nitrogen measures the amount of urea nitrogen in the blood similar to phosphorus being indicative of blood phosphorus concentration. Creatinine is broken down protein from normal catabolism of muscle and food. The values being compared are averaged from within four weeks before treatment and four weeks post-treatment. The patients used in this study underwent common clinical treatments for chronic kidney disease in addition to the adipose-derived stem cell therapy which will be referred to generally as AD-MSC therapy, stem cell therapy, or therapy hereafter. It was used once in the majority of patients except for three canines and one feline that received two treatments. However, due to the lack of additional data, I will only be evaluating the effect of the first stem cell therapy. The additional treatments were given after the time parameters acknowledged previously and do not impact the data being evaluated. Patients were given intravenous fluids (unspecified amounts, relative to body weight and dehydration status) as fluid diuresis. Diet changes were advised, such as the Royal Canin Renal diet. Additional vitamin and mineral supplements, usually Lixotinic, were also used. A renal support drug, namely Azodyl, was also used in every patient.

For this study, chemistry panel data from eleven canine patients and four feline patients were used. All personally identifying information was removed and therefore each patient has been assigned a specific number to their patient history along with the designation of species. This data is taken from a general practice clinic so there is a lack of total uniformity that might be attained in a research facility. As such, I acknowledge that while all clients were advised to make diet changes and administer

the prescribed medications, there is no way to be certain of what directives were followed and to what degree. However, the timeline and in-clinic treatment of each patient is very similar which provides added validation to the results that may be observed from the stem cells. Each participant but one has at least one chemistry panel run within four weeks before stem cell therapy is administered, but some have up to four in that time. Typically another test is run as close two weeks as possible after the treatment to allow it time to take effect. However, this was not always available for each patient. Therefore, all data used was collected as early as one week post-operatively, except canine patient two, who only had postoperative panels at the two and five day marks. The reference values used for blood urea nitrogen, creatinine, and phosphorus are taken from the Abaxis VetScan VS2, the analyzer used for all patients in the study. All three values are measured in milligrams per deciliter (mg/dL). The canine reference intervals are as follows: blood urea nitrogen of 7-25 mg/dL, creatinine of 0.3-1.4 mg/dL, and phosphorus of 2.9-6.6 mg/dL. The feline reference ranges are as follows: blood urea nitrogen of 10-30 mg/dL, creatinine of 0.3-2.1 mg/dL, and phosphorus of 3.4-8.5 mg/dL. The number of chemistry panels, including kidney profiles, after AD-MSC therapy varied, some with only one and others up to six. All data was averaged within its group, with panels taken on the day of treatment being grouped with the before section.

A brief description of each patient's clinical chemistry values, comorbidities, or other important information such as additional treatment are provided below along with Table 1 that lists the average BUN, CRE, and PHOS before and after treatment. Mean survival time and age of death are noted for the canines. When examining these averages it is pertinent to remember that important factors such as breed and weight

are unknown and comorbidities vary between patients. All of these factors may affect those means positively or negatively.

Table 1
Pre/Post Mean Chemistry Panel Values

Patient	BUN Pre-	BUN Post-	CREA Pre-	CREA Post-	PHOS Pre-	PHOS Post-
Canine 1	83.5	48	3.75	10.6	12.8	5
Canine 2	170	40.5	12.8	1.9	18	4.7
Canine 3	71	79.2	2.1	3.27	6.4	7.8
Canine 4	42	29	2.1	2.55	4.5	5.2
Canine 5	34	19	1.8	1	4.2	4.4
Canine 6	112.5	34	3.6	1.3	9.5	4.3
Canine 7	109	44	1.7	1.9	5.9	6.3
Canine 8	156.5	173	1.9	2.1	6.85	6.5
Canine 9	104.5	111.5	3.25	2.95	7.55	6.9
Canine 10	53	34	2.27	1.9	5.63	4.8
Canine 11	75.25	63.5	3.95	3.55	10.53	6.1
Feline 1	84	67	4.1	3.1	9.4	8.1
Feline 2	225.5	105	19.4	9.1	21.15	12.8
Feline 3	85	183	7	13.2	6.8	11.45
Feline 4	96	89	5.95	5	5.9	8.5

Canine 1 had two panels taken before therapy and one noted ten days after, while additionally managing hypothyroidism. It received another stem cell treatment twenty months post initial therapy and survived twenty-three months after administration of the first stem cell therapy. It was euthanized at twelve years old.

Canine 2 had two panels taken before therapy, but one was not provided, with two panels taken two and five days after. The patient was euthanized seven days after administration of stem cell therapy at seven years old.

Canine 3 yielded two panels before and six panels after treatment. Those after treatment were one each at one and two weeks and the other four after three weeks post-operatively. This patient received a second round of AD-MSC therapy one month after the first and was euthanized seven months following the second at ten years old.

Canine 4 gave two panels prior to treatment, as well as two after (eleven days and three weeks respectively). A second treatment was given nine months after the initial and the patient was euthanized six months later at eight years old.

Canine 5 gave one panel at each designation, with the second being taken two weeks after therapy. This patient was managing an unspecified heart condition as indicated by the prescribed diuretic furosemide in the patient's treatment history. Canine 5 was euthanized thirteen months after stem cell therapy aged fourteen years old.

Canine 6 had two panels before and one taken two weeks after with no noted comorbidities. It was euthanized one year and two months after its solitary treatment at age eleven.

Canine 7 had one profile before and one three weeks after treatment, but the profile run before was too hemolyzed for its creatinine and phosphorus values to be obtained. The hemolyzation was likely due to the heart condition suffered in addition to the kidney disease. Canine 7 was euthanized one year after treatment and was fifteen years old.

Canine 8 had two profiles before stem cell therapy, but its next post-operative panel was recorded six weeks later. Much like the previous patient, this one also was suffering from a heart condition at the time of treatment. It died seven weeks after treatment at twelve years old.

Canine 9 provided two panels before and two after, with those after coming at two and four weeks post therapy. It survived three months after receiving stem cells and was euthanized at ten years old.

Canine 10 had three profiles prior to treatment and one two weeks after. It was specifically noted here that the animal initially refused the advised renal diet when offered in place of its previous diet, but eventually were able to adjust the patient to the new one. It was euthanized at thirteen years old, one year and three months after treatment.

Canine 11 had four panels before stem cell therapy, with two after at the two and four week marks. This patient survived another nine months before being euthanized at eleven years old.

Each canine's mean survival time and age at death was calculated with the average patient surviving 8.91 months after receiving stem cell therapy for chronic kidney disease. The average age of death for canine patients in this study was 11.18 years old.

Feline 1 had one profile before and one profile at a two week checkup. This patient had no noted comorbidities and was deceased at eleven years old, three months after treatment.

Feline 2 provided two panels prior and two post-treatment. One was taken one week after and the other at three weeks. One panel from each category has blood urea nitrogen measured above 180, the highest the analyzer could notate in this case, as well as creatinine above 20 in the same after AD-MSC therapy panel. This feline was euthanized two months after treatment at nine years old.

Feline 3 had one panel before and two after, with the two week post-stem cell panel having the same extremely elevated BUN reading as feline 2. The four week after therapy profile had values back within readable range. It was noted that this patient was frequently anemic but for an unspecified reason. There is no additional information given in the patient's record of other comorbidities. It survived for eight months and died at two years old. The date between the last clinic visit and date of death are six months apart, meaning cause of death is unknown and unable to be inferred from patient history or doctor's notes.

Feline 4 had two panels prior to treatment and one a week after. This patient's notes end six months from the stem cell therapy with no noted date of death, likely meaning this client began going to a different clinic, moved away, or for some other reason not related to pet health discontinued their care at this clinic.

Analysis

A single factor analysis of variance was completed for each of the chosen chemistry panel values and separated by species, yielding six individual ANOVAs that are summarized in Tables 2-9 in the List of Tables and Figures. After running each test, only canine phosphorus was found to be statistically significant.

Discussion

Before analyzing the data I proposed a null and alternative hypothesis. My null hypothesis was that stem cell therapy would have no statistically significant effect on the patients' chemistry panel values. My alternative hypothesis was that stem cell therapy would have a statistically significant effect on patients' chemistry panel values. A single factor ANOVA test was run for each chemistry panel value with a post-hoc t-test (for two samples assuming unequal variances) following if the data was found to be statistically significant. The eta-squared value indicates effect size; 0.01-0.06 is a small effect, 0.06-0.14 is moderate, and greater than 0.14 is a large effect. The probability of type 1 error was set to be 10%. The inferences from each ANOVA has been included, even if not found to be statistically significant. The percent of change attributed to stem cell therapy, where applicable, assumes no other interferences. Table 1 in the Background section contains the chemistry panel averages from before and after treatment for both species and is reported in milligrams per deciliter.

It is necessary to acknowledge some limitations and alternative options utilized when analyzing the given data. In the case of canine 7, it was elected to use a panel from three months prior to treatment as it was the most recent panel available. This could potentially affect the data, but because the number of patients was already limited it was elected to keep this oddity in the study. Similarly, with feline 2 and 3 both had values well outside normal range that was read as "greater than 180" on the chemistry profile. This number is already excessively elevated out of normal ranges so 181 was used as a stand-in when there was no specific number above 180 given. The second post-treatment panel (three weeks after) of cat 2 also has a creatinine concentration of

greater than 20, with 21 being used there in place of the unknown value. However, despite these inconsistencies each patient had another panel that could be averaged with the assumed value to minimize the impact the unknown number might have on the outcome of the ANOVA tests.

Canine Results

The results from the eleven canine patients are summarized in Table 2 and each individual ANOVA run was organized into separate tables. The blood urea nitrogen ANOVA test (Table 3) was not statistically significant with $F(1, 20) = 2.57$, $p = 0.12$. The eta-squared value for canine BUN was calculated to be 0.11 suggesting moderate effect size. A positive mean difference was also observed, allowing us to attribute roughly 11.4% of the favorable change to the stem cell therapy. The creatinine ANOVA test (Table 4) was not statistically significant with $F(1, 20) = 0.21$, $p = 0.66$ with a positive mean difference and an eta-squared of 0.010. This yields a small positive effect size, making 1% attributable to the therapy. Phosphorus exhibited a large positive effect with the ANOVA reporting $F(1,20) = 4.4$, $p = 0.05$, eta-squared of 0.18 and a positive mean difference. (Table 5) The p-value indicates statistical significance for the improvement in phosphorus values.

Table 2
Canine Mean Summary

Canine Mean Summary	Count	Mean	Standard Deviation
BUN Before	11	91.93	43.92
BUN After	11	61.43	45.34
BUN Mean Difference		**30.5**	
CREA Before	11	3.57	3.18
CREA After	11	3	2.64
CREA Mean Difference		**0.57**	
PHOS Before	11	8.35	4.14
PHOS After	11	5.64	1.15
PHOS Mean Difference		**2.71**	

Table 3
Canine BUN ANOVA

ANOVA	F-Statistic	P-Value	eta-squared
Canine BUN	2.57	0.12	0.11

Table 4
Canine CREA ANOVA

ANOVA	F-Statistic	P-Value	eta-squared
Canine CREA	0.2	0.66	0.01

Table 5
Canine PHOS ANOVA

ANOVA	F-Statistic	P-Value	eta-squared
Canine PHOS	4.4	0.05	0.18

Feline Results

The four feline patients' data was organized in the same manner as the canines, with Table 6 containing the mean summaries for each profile value. The ANOVA for feline blood urea nitrogen (Table 7) was not statistically significant with $F(1, 6) = 0.074$, $p = 0.79$. An eta-squared value of 0.012 and a positive mean difference were observed, a small positive effect. Creatinine's test (Table 8) yielded $F(1, 6) = 0.13$ and $p = 0.73$, meaning it is not statistically significant. A small effect size was noted with the eta-squared value calculated to be 0.022 with a positive mean difference. Phosphorus (Table 9), like the previous two values, was not found to be statistically significant with $F(1, 6) = 0.026$, $p = 0.88$. It exhibited an eta-squared value of 0.0044 meaning there is not a significant effect size measured as well.

Table 6
Feline Mean Summary

Feline Mean Summary	Count	Mean	Standard Deviation
BUN Before	4	122.63	68.8
BUN After	4	111	50.46
BUN Mean Difference		**11.63**	
CREA Before	4	9.11	6.96
CREA After	4	7.6	4.5
CREA Mean Difference		**1.5**	
PHOS Before	4	10.81	7.05
PHOS After	4	10.21	2.28
PHOS Mean Difference		**0.6**	

Table 7
Feline BUN ANOVA

ANOVA	F-Statistic	P-Value	eta-squared
Feline BUN	0.074	0.79	0.012

Table 8
Feline CREA ANOVA

ANOVA	F-Statistic	P-Value	eta-squared
Feline CREA	0.13	0.73	0.022

Table 9
Feline PHOS ANOVA

ANOVA	F-Statistic	P-Value	eta-squared
Feline PHOS	0.026	0.88	0.0044

Conclusion

Despite the majority of the ANOVA tests returning as not statistically significant, the data shows promise for future studies. The four tests of canine blood urea nitrogen, feline blood urea nitrogen, canine creatinine, and feline creatinine all expressed positive effects and thus would reject the null hypothesis. Canine phosphorus exhibited a positive mean difference, statistical significance, and a large effect size, rejecting the null hypothesis. Feline phosphorus was the only value that was both not statistically significant and did not exhibit an eta-squared of qualitative value. As such, it failed to reject the null hypothesis. It would be beneficial to run a larger sample size through the analysis to attempt to definitively display a significant link between the stem cell therapy and the improved chemistry panel values. The improvements in diagnostic methods between the time these patients were treated and now would allow researchers to

diagnose chronic kidney disease earlier. Therapies started when diagnosed by creatinine and blood urea nitrogen are typically undergone in stages three or four. Once the disease has reached this level of progression its effects are much harder to treat. Thus, it would be reasonable to test adipose-derived stem cell therapy in earlier stages of CKD using a value such as symmetrical dimethylarginine as the diagnostic tool. This would allow veterinarians to attempt to halt the disease's progression in a less severe state and likely improve both the prognosis and quality of life of the patient.

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