Acta Scientific Veterinary Sciences (ISSN: 2582-3183)

Volume 7 Issue 11 November 2025

Research Article

# Differences in MicroRNA Expression in Aqueous Humor of Dogs Predisposed to Developing Primary Angle Closure Glaucoma: A Pilot Study

**Hans D Westermeyer*, Jacklyn H Salmon and Michala de Linde Henriksen**

*North Carolina State University, College of Veterinary Medicine, Department of Clinical Sciences, Raleigh, NC*

***Corresponding Author:** Hans D Westermeyer, North Carolina State University, College of Veterinary Medicine, Department of Clinical Sciences, Raleigh, NC.

**Received:** September 08, 2025

**Published:** October 06, 2025

## Abstract

**Purpose:** The purpose of this pilot study was to explore the possibility of a common molecular pathway leading to the development of primary angle closure glaucoma (PACG) in dogs. This was done by comparing the aqueous humor (AH) microRNA (miRNA) profile of dogs predisposed to developing PACG to that of normal healthy dogs.

**Material and Methods:** The AH (250µl) samples were collected under general anesthesia from the normal eye of dogs that had been diagnosed with PACG associated intraocular pressure (IOP) elevation in the opposite eye (study group; n=3 eyes). Aqueous humor from normal healthy eyes was also collected from purpose bred, beagle dogs immediately after euthanasia (control group; n=3 eyes). The AH samples were submitted to a commercial laboratory for miRNA sequencing and analysis.

**Results:** MicroRNA was successfully sequenced from all six AH samples. There were 19 sequences (16 novel and 3 known) which showed differential expression (13 upregulated and 6 downregulated) between eyes of the study group and the control group ($P < 0.05$). There were three sequences that were expressed in all study group dogs but not in any of the control group dogs (cfa-miRNA-206, NovelmiRNA-222, and NovelmiRNA-366).

**Conclusions:** There may be differences in miRNA expression in dog eyes predisposed to developing PACG prior to the onset of IOP elevation. Further investigation of these differences may lead to the discovery of the molecular mechanisms involved in IOP elevations in dogs with PACG and the development of novel diagnostic and treatment modalities.

**Keywords:** MicroRNA Expression; Aqueous Humor; Predisposed; Glaucoma

## Introduction

Primary angle closure glaucoma (PACG) is the most common form of primary glaucoma in the dog.[1] The prevalence of primary glaucoma across all dog breeds is nearly 1%. However, in breeds in which the disease is most common, such as the American cocker spaniel, Basset hound, Chow Chow, and Shar-Pei, prevalence is greater than 4% [1]. The disease is typically associated with sudden, severe, painful rises in intraocular pressure (IOP) that lead to vision loss if not addressed immediately. Affected dogs show characteristic pectinate ligament abnormalities, yet not all dogs

**Citation:** Westermeyer HD*., et al.* "Differences in MicroRNA Expression in Aqueous Humor of Dogs Predisposed to Developing Primary Angle Closure Glaucoma: A Pilot Study". *Acta Scientific Veterinary Sciences* 7.11 (2025): 03-09.

with these abnormalities develop glaucoma [2]. The mechanism by which the pectinate ligament changes are linked to the onset of glaucoma or the molecular mechanism which leads to elevated IOPs has not been discovered.

Previous attempts to identify these mechanisms have centered around the investigation of genetic mutations in dogs with PACG [3]. Genetic mutations associated with the presence of glaucoma have been discovered in several breeds. However, these mutations are only associated with disease within a specific breed, and in some cases, only within a specific sub-population of a breed [4,5]. For example, a mutation in NEB was associated with glaucoma in a small group of Bassett hounds but this association did not remain when investigated in a broader cohort [4]. This has led investigators to wonder whether PACG is a polygenic trait with environmental influence [2,4]. Considering the similarities in pectinate ligament abnormalities and disease progression among dogs of different breeds affected with PACG, perhaps the multiple genes and environmental factors feed into a common molecular pathway that causes disease across all breeds. Discovery of this final molecular pathway could lead to the identification of the multiple genes that feed into it, leading to new ways to treat and prevent the disease.

Unpublished data from our lab suggests there is elevation of aqueous humor (AH) inflammatory cytokine levels of interleukin-6, interleukin-8, and monocyte chemoattractant protein-1 across multiple breeds predisposed to PACG *prior* to IOP elevation, implying there are differences in AH composition in dogs with PACG that might lead to rises in IOP. We hypothesized these differences might be driven by differences in local gene expression modulated by microRNA within AH. MicroRNA (miRNA) are short, single-stranded, non-coding RNA molecules that lead to markedly different local gene expression within an environment. This effect is mediated by miRNA binding to complementary sequences within messenger RNA molecules which results in decreased messenger RNA translation, thus altering local protein expression [6]. miRNA is found in human AH and has been shown to play a role in the development of different types of glaucoma in humans [6]. This may also be true in dogs. However, the feasibility of sequencing miRNA from canine AH has not been reported. Therefore, the goal of this exploratory pilot study was twofold: 1) determine whether miRNA could be sequenced from canine AH and 2) compare the microRNA profiles of dogs predisposed to PACG and normal healthy dogs.

## Material and Methods

This was an exploratory prospective controlled pilot study. Client owned dogs presenting to the veterinary hospital with overt IOP elevations caused by PACG in one eye and requiring surgery to control pain or preserve vision in that eye were eligible to participate in the study. Participation in the study consisted of collection of 250uL of AH from the opposite normal eye under general anesthesia. Informed consent was obtained from all owners, and all procedures were approved by the Institutional Animal Care and Use Committee (Protocol # 21-146).

### Study animals

Inclusion criteria for dogs with PACG (study group) consisted of documented IOP elevation in one eye >35mmHg, absence of intraocular disease known to cause secondary glaucoma, and pectinate ligament and ciliary cleft abnormalities associated with PACG. The presence of PACG was confirmed on a full ophthalmic evaluation by a board-certified veterinary ophthalmologist (HDW). All PACG dogs had severe pectinate ligament abnormalities documented photographically with a wide-angle contact viewing system (RetCam II, Clarity Medical Systems, Pleasanton, CA) and ciliary cleft morphology evaluated with high resolution ultrasound (UHF70, VevoMD, Fujifilm, Bothell, WA).

The normal healthy dogs (control group) consisted of purpose-bred, beagle dogs undergoing euthanasia for an unrelated study. These dogs had not received any systemic medication or undergone any surgical procedures during the previous six months and had a normal ophthalmic examination documented by a board-certified veterinary ophthalmologist (HDW). The AH was collected immediately following euthanasia. The globe was then enucleated and hemisected under an operating microscope. Normal pectinate ligament morphology and normal ciliary cleft morphology was confirmed by direct observation by a board-certified veterinary ophthalmologist (HDW).

### Aqueous humor collection

The AH was collected from dogs in the study group while under general anesthesia using aseptic technique and immediately after euthanasia from dogs in the control group. The AH was collected by inserting a 30g x 1/2" hypodermic needle into the anterior chamber using a standard limbal paracentesis approach. The AH was then allowed to passively drip from the hub of the needle into a microcentrifuge tube until at least 250uL had accumulated. The AH was then immediately frozen at -80$^{o}$C and kept frozen until analysis. Topical neomycin/polymyxin/dexamethasone 0.1% ophthalmic suspension (Bausch + Lomb, Bridgewater, NJ) was prescribed for dogs in the study group following aqueocentesis (1 drop every 8 hours for one week).

### MicroRNA analysis

The AH samples were submitted to a commercial laboratory for high throughput sequencing of small RNA sequences of 18-30 base pairs (GENEWIZ, Azenta Life Sciences, South Plainfield, NJ) using their standard analysis package. Analysis workflow as described by the laboratory was as follows: Raw reads obtained from microRNA sequencing were processed by removing contamination and adapter sequences. Statistical analysis on lengths and counts of the filtered reads as well as data volume was performed as part of data quality control. The filtered reads were aligned to the miRbase database composed of known miRNA sequences. This was followed by miRNA annotation. Additionally, the reads were aligned to the Rfam database to study non-coding RNA distribution. For novel miRNA prediction, the sequences were aligned to the genome of the domestic dog and subjected to RNA folding and secondary structure analysis.

### Statistical analysis

A negative binomial general linear model fitted to the normalized sequence counts was used to compare the expression of miRNA sequences from the study group to the control group. Then a quasi-likelihood F-test was performed for each sequence to determine its differential expression. Significance was set at $P < 0.05$. A correction for multiple testing was carried out using the Benjamini-Hochberg method to control the false discovery rate. Analysis was performed using R and the Bioconductor package EdgeR (V3.4.6, www.bioconductor.org).

## Results

MicroRNA was successfully sequenced from all AH samples collected from dogs in the study group (n=3 eyes, Dogs 1-3) and in the control group (n=3 eyes, Dogs 4-6). Figure 1 shows a goniophotograph of a representative quadrant and a high-resolution ultrasound of the ciliary cleft from the same quadrant from each study group dog. Dog 1 was a 14-year-old, male neutered, Bassett hound with a nearly complete sheet of uveal tissue covering the iridocorneal angle (occlusion) and a nearly entirely collapsed ciliary cleft. Dog 1 died 6 months following sample collection due to unrelated, age associated disease. Dog 2 was a 3.5-year-old, female spayed, Siberian Husky that also had a nearly complete sheet of uveal tissue covering the iridocorneal angle (occlusion) and a nearly normal ciliary cleft. Dog 2 continues to be monitored every three months and has not developed IOP elevations in the sampled eye 41 months later. Dog 3 was a 7-year-old, female spayed, labradoodle that had a sheet of abnormal uveal tissue covering approximately the bottom 40% of the iridocorneal angle (lamina) and a narrow ciliary cleft opening. Dog 3 developed IOP elevation, glaucoma, and vision loss in the sampled eye 3.8 months after sample collection. The control group (dogs 4-6) was made up of three 2-year-old purpose bred beagle dogs (two males and one female, all intact).

Differential expression of miRNA was noted for 19 miRNA sequences ($P < 0.05$, Table 1). There were 13 sequences that were upregulated and 6 sequences that were downregulated in the study group compared to the control group. However, the false discovery rate for all of these was greater than 89% (higher than the accepted <5%) [7]. There were three miRNA sequences (cfa-miR-206, NovelmiRNA-222, NovelmiRNA-366) that were expressed in AH of all dogs in the study group (Dog 1-3) but not in any of the dogs from the control group (Dog 4-6).

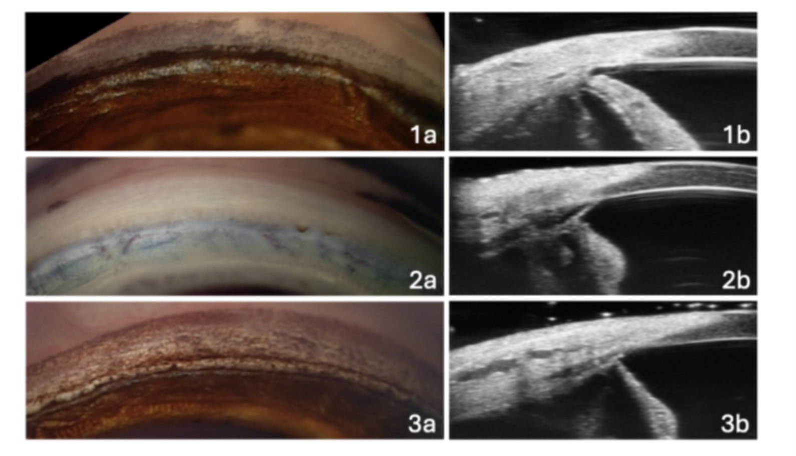

**Figure 1:** Representative goniophotographs of the iridocorneal angle (a panels) and high-resolution ultrasound (HRUS) images of the ciliary cleft (b panels) from the three eyes (Dog 1-3) predisposed to primary angle closure glaucoma (PACG) (study group) sampled in this study. There are marked pectinate ligament abnormalities visible in all 3 goniophotographs, with Dog 1 and 2 displaying a nearly solid sheet of tissue covering the opening of the ciliary cleft and a solid sheet covering approximately 40% of the opening in Dog 3. The ciliary cleft is nearly collapsed in Dog 1, nearly completely normal in Dog 2, and partially collapsed at the opening in Dog 3. A hyperechoic linear structure, representing the pectinate ligament abnormality seen gonioscopically can be seen at the opening of the ciliary cleft in Dog 2 and 3 in the HRUS images.

## Discussion

The current study shows it is possible to sequence miRNA from AH of dogs and hints at the possibility of differential expression of these non-coding sequences in dogs with PACG (study group) compared to dogs unaffected with glaucoma (control group). Since these differences were detected in eyes predisposed to developing glaucoma prior to any IOP elevations and clinical signs of glaucoma they are much more likely to represent a causal mechanism for IOP elevation, as opposed to a downstream effect of IOP elevation.

This study is novel in that it is the first to show differences in miRNA expression in AH from dogs that are predisposed to glaucoma *prior* to any elevations in IOP. There are several studies evaluating AH cytokines in PACG [8-10]. However, these studies have all evaluated the AH cytokines *after* the onset of overt clinical signs of glaucoma, and in most cases during the end-stage disease when the eye is blind, painful, and requires enucleation. The changes seen in these end-stage eyes most likely do not represent the inciting mechanisms that lead to IOP elevation, but instead the downstream effects of the ischemia and inflammation associated with the dramatic and sustained IOP rises seen in canine PACG [11,12]. Although these findings are important and point to the pathology *caused* by IOP rises, they are unlikely to help determine the mechanisms that lead to the rises in IOP. To determine the mechanisms that lead to the IOP rises, the differences in AH composition *prior* to those IOP rises occurring must be evaluated. Cytokine profiles of beagles with ADAMTS10-associated POAG have not been shown to be different to non-mutant beagles and do not appear to be impacted by IOP [13]. The present study shows detectable differences in miRNA in AH of dogs predisposed to PACG prior to IOP elevations. This discovery represents an extremely important data point and may represent a causal association for the development of elevated IOP.

| Gene ID | Sequence length | TPM D1 | TPM D2 | TPM D3 | TPM D4 | TPM D5 | TPM D6 | logFC | logCPM | *P* | FDR | Regulation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NovelmiRNA-36 | 23 | 0 | 67696.19 | 0 | 0 | 0 | 0 | 14.11374 | 13.5436 | 0.00414 | 0.89937 | Up |
| cfa-miR-206 | 20 | 589.54 | 42.02 | 2020.74 | 0 | 0 | 0 | 9.48231 | 8.972374 | 0.004581 | 0.89937 | Up |
| NovelmiRNA-349 | 24 | 0 | 0 | 50092.57 | 0 | 0 | 0 | 13.82006 | 13.25056 | 0.00563 | 0.89937 | Up |
| NovelmiRNA-270 | 20 | 0 | 28500.89 | 0 | 0 | 0 | 0 | 12.86576 | 12.29916 | 0.007838 | 0.89937 | Up |
| NovelmiRNA-372 | 24 | 0 | 0 | 0 | 0 | 14209.32 | 0 | -12.0721 | 11.5097 | 0.008496 | 0.89937 | Down |
| NovelmiRNA-295 | 19 | 0 | 1376.19 | 1627.52 | 0 | 0 | 0 | 9.699617 | 9.181207 | 0.009537 | 0.89937 | Up |
| NovelmiRNA-241 | 19 | 0 | 15012.08 | 0 | 0 | 0 | 0 | 11.94101 | 11.37988 | 0.011473 | 0.89937 | Up |
| NovelmiRNA-366 | 18 | 675.81 | 462.23 | 436.92 | 0 | 0 | 0 | 8.58803 | 8.129845 | 0.011992 | 0.89937 | Up |
| NovelmiRNA-38 | 18 | 1437.9 | 0 | 906.6 | 0 | 0 | 0 | 9.112666 | 8.620656 | 0.019227 | 1 | Up |
| NovelmiRNA-351 | 18 | 0 | 0 | 0 | 0 | 3391.11 | 0 | -10.0074 | 9.477369 | 0.024906 | 1 | Down |
| NovelmiRNA-266 | 18 | 0 | 0 | 0 | 0 | 3391.11 | 0 | -10.0074 | 9.477369 | 0.025695 | 1 | Down |
| NovelmiRNA-181 | 19 | 0 | 0 | 0 | 0 | 0 | 5443.43 | -9.94419 | 9.415266 | 0.026595 | 1 | Down |
| cfa-miR-142 | 21 | 0 | 567.29 | 1015.83 | 0 | 0 | 0 | 8.791651 | 8.319341 | 0.030788 | 1 | Up |
| NovelmiRNA-275 | 19 | 0 | 0 | 3222.27 | 0 | 0 | 0 | 9.863829 | 9.340187 | 0.032033 | 1 | Up |
| NovelmiRNA-48 | 19 | 0 | 0 | 0 | 2618.38 | 0 | 0 | -9.50252 | 8.986569 | 0.040441 | 1 | Down |
| NovelmiRNA-65 | 19 | 0 | 0 | 0 | 2618.38 | 0 | 0 | -9.50253 | 8.986569 | 0.040736 | 1 | Down |
| NovelmiRNA-222 | 21 | 201.31 | 336.17 | 322.23 | 0 | 0 | 0 | 7.799999 | 7.419539 | 0.043462 | 1 | Up |
| NovelmiRNA-155 | 18 | 848.36 | 0 | 720.91 | 0 | 0 | 0 | 8.572982 | 8.116244 | 0.044372 | 1 | Up |
| cfa-miR-143 | 21 | 15687.46 | 7185.63 | 265012.2 | 2178.31 | 14086.14 | 7053.46 | 3.72978 | 15.84821 | 0.047174 | 1 | Up |

**Table 1:** The Gene IDs, sequence length, and normalized transcripts per million (TPM) of the 19 differentially expressed sequences from aqueous humor samples of the study group (Dog 1-3) and the control group (Dog 4-6) are shown in the first 8 columns. LogFC represents the $\log_2$ fold change, logCPM represents the average $\log_2$ counts per million, *P* represents the calculated probability of differential expression from a negative binomial general linear model fitted to the normalized sequence counts, and FDR represents the false discovery rate (calculated by adjusting *P* according to the Benjamini-Hochberg procedure). Up- or down-regulation of each sequence is noted in the last column.

Most of the sequences found to have differential expression were novel sequences. As such their impact on gene expression has not been determined and their downstream effects on molecular mechanisms cannot yet be elucidated. There were three known miRNA sequences identified among sequences that were upregulated in dogs with PACG: cfa-miR-142, cfa-miR-143, and cfa-miR-206. cfa-miR-142 corresponds to a sequence found on chromosome 9 and has previously been shown to be downregulated in dogs with congestive heart failure secondary to myxomatous mitral valve degeneration [14]. cfa-miR-143 has previously been shown to stimulate activation of p53 and caspase3 to induce apoptosis in canine cells infected with canine influenza virus [15]. cfa-miR-206 corresponds to a sequence found on chromosome 12 that has previously been shown to affect cardiac autonomic nerve remodeling [16] and upregulated in peripheral blood mononuclear cells of dogs infected with *Leishmania infantum* [17]. The cfa-miR-206 sequence was one of the sequences that was expressed only in dogs with PACG. What influence these sequences have on gene expression and molecular processes in the trabecular meshwork is yet to be determined and represents the next step necessary for the discovery of the molecular mechanisms responsible for IOP elevation in PACG.

The limitations of this study are the small sample size and imperfect control group. As analysis of genomic data requires the comparison of expression levels of potentially hundreds or thousands of sequences, correcting for multiple comparisons is imperative. In the present study, the raw $P$ values ($P < 0.05$) identified 19 sequences with differential expression. However, when corrected for multiple comparisons, none of these reached the generally accepted false discovery rate of < 5% threshold [7]. As this was an exploratory pilot study with a small sample size, it was not unexcepted. It is reasonable to assume that if our results were extended to additional eyes, the statistical significance of our data would increase and many of these differences would surpass the threshold.

All the dogs in the control group were male and female intact, while all the dogs in the study group were neutered or spayed. Moreover, all the dogs in the control group were young beagle dogs, while the dogs in the study group were generally much older and of three different breeds. It is possible the differences detected represent differences in expression due to hormonal, age, or breed differences. However, studies of human aqueous humor have failed to identify differences in cytokine expression attributable to age [18], race [19], or sex [20]. Divergence in expression of circulating miRNA sequences have been detected in humans according to age, ethnic groups, or hormonal levels [21]. Thus it is possible for the differences between the age and neuter status of the study group versus the control group to have an impact on the expression of AH molecules in dogs.

## Conclusion

The data from this study documents the feasibility of sequencing miRNA from canine AH. The study also suggests there likely are differences in the AH miRNA profiles of dogs with PACG prior to the onset of IOP elevations when compared to AH from normal healthy dogs. Further confirmation and characterization of these differences with larger studies holds great potential to reveal the molecular mechanisms that lead to IOP elevations in PACG and should be pursued. Defining the molecular mechanisms that lead to pressure elevations could lead to novel diagnostic and treatment methods for canine PACG.

### Data Availability

The entire data set supporting this report is available upon request from the corresponding author.

### Funding Statement

This study was funded by an intramural seed grant from the North Carolina State University College of Veterinary Medicine and by a grant from the Vision for Animals Foundation (VAFGL-2017).

## Bibliography

1. Gelatt KN and MacKay EO. “Prevalence of the breed-related glaucomas in pure-bred dogs in North America”. *Veterinary Ophthalmology* 7 (2004): 97-111.

2. Komáromy AM and Petersen-Jones SM. “Genetics of Canine Primary Glaucomas. *Veterinary Clinics of North America: Small Animal Practice* 45 (2015): 1159-1182.

3. Graham KL., *et al*. “Genetic and Biochemical Biomarkers in Canine Glaucoma”. *Veterinary Pathology* 54 (2016): 194-203.
4. Ahram DF., *et al*. “Variants in Nebulin (NEB) Are Linked to the Development of Familial Primary Angle Closure Glaucoma in Basset Hounds”. *PLoS One* 10 (2015): e0126660.
5. Kanemaki N., *et al*. “Dogs and Humans Share a Common Susceptibility Gene SRBD1 for Glaucoma Risk”. *PLoS One* 8 (2013): e74372.
6. Greene KM., *et al*. “The role of microRNAs in glaucoma”. *Experimental Eye Research* 215 (2022): 108909.
7. Benjamini Y and Hochberg Y. “Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing”. *Journal of the Royal Statistical Society: Series B (Methodological)* 57 (1995): 289-300.
8. Durieux P., *et al*. “Tumor necrosis factor-alpha concentration in the aqueous humor of healthy and diseased dogs: A preliminary pilot study”. *Journal Français d’Ophtalmologie* 38 (2015): 288-294.
9. Weinstein WL., *et al*. “Identification of ocular matrix metalloproteinases present within the aqueous humor and iridocorneal drainage angle tissue of normal and glaucomatous canine eyes”. *Veterinary Ophthalmology* 10 (2007): 108-116.
10. Terhaar HM., *et al*. “Pro-inflammatory cytokines in aqueous humor from dogs with anterior uveitis and post-operative ocular hypertension following phacoemulsification, primary glaucoma, and normal healthy eyes”. *PLoS One* (2022): 17.
11. Scott EM., *et al*. “Early histopathologic changes in the retina and optic nerve in canine primary angle-closure glaucoma”. *Veterinary Ophthalmology* 16.1 (2013): 79-86.
12. Fick CM and Dubielzig RR. “Short posterior ciliary artery anatomy in normal and acutely glaucomatous dogs”. *Veterinary Ophthalmology* (2016): 43-49.
13. Collins E., *et al*. “Pro-Inflammatory Cytokines in Aqueous Humor From ADAMTS10-Mutant Beagles at an Early Stage of Open-Angle Glaucoma”. *Veterinary Ophthalmology* (2025): 1-9.
14. Jung SW and Bohan A. “Genome-wide sequencing and quantification of circulating microRNAs for dogs with congestive heart failure secondary to myxomatous mitral valve degeneration”. *American Journal of Animal and Veterinary Sciences* 79 (2018): 163-169.
15. Zhou P., *et al*. “cfa-miR-143 Promotes Apoptosis via the p53 Pathway in Canine Influenza Virus H3N2-Infected Cells”. *Viruses* 9 (2017): 360.
16. Zhang Y., *et al*. “MicroRNA Profiling of Atrial Fibrillation in Canines: MiR-206 Modulates Intrinsic Cardiac Autonomic Nerve Remodeling by Regulating SOD1”. *PLoS One* 10 (2015): e0122674.
17. Soares MF., *et al*. “Differential expression of miRNAs in canine peripheral blood mononuclear cells (PBMC) exposed to Leishmania infantum *in vitro*”. *Research in Veterinary Science* 134 (2021): 58-63.
18. Peng C., *et al*. “Characteristic Cytokine Profiles of Aqueous Humor in Glaucoma Secondary to Sturge-Weber Syndrome”. *Frontiers in Immunology* 11 (2020): 480347.
19. Trivedi RH., *et al*. “Influence of Race and Age on Aqueous Humor Levels of Transforming Growth Factor-Beta 2 in Glaucomatous and Nonglaucomatous Eyes”. *Journal of Ocular Pharmacology and Therapeutics* 27 (2011): 477.
20. Haq Z., *et al*. “Sex-Based Analysis of Potential Inflammation-Related Protein Biomarkers in the Aqueous Humor of Patients With Diabetes Mellitus”. *Translational Vision Science and Technology* 10 (2012): 12.
21. Ferreira T., *et al*. “Exploring the role of microRNAs as diagnostic and prognostic biomarkers in canine mammary tumors”. *GeroScience* 46 (2024): 6641-6657.